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ARTICLE
Microbial Ecology

Tripled plant productivity and soil microbiome restructuring in a greenhouse volcanic ash fertilization experiment

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Abstract

Volcanic ash is widely held responsible for fertilizing soils, but the processes and conditions leading to volcanic soil fertility remain poorly understood. We report here the results of a greenhouse volcanic ash fertilization (VAF) experiment aimed to explore the impact of basaltic ash addition (0–10 wt%) to soil on plant growth, nutrient uptake, and the soil microbiome. Our four-month experiment with the Coleus species *Plectranthus scutellarioides* on forest soil revealed a non-linear growth response with distinct growth patterns below and above 3 wt% ash addition, respectively. Low ash VAF (<3 wt% ash) had a negligible growth impact, while high ash addition yielded a threefold increase in biomass production, reproductive effort, and total nutrient uptake in aboveground plant biomass. An increased uptake of nutrients that are not part of the ash itself (e.g., nitrogen) implies that VAF was not a direct nutrient addition process but rather acts indirectly. The soil microbiome composition (16S rRNA; Bacteria/Archaea, and 18S rRNA; Eukaryota, rRNA gene markers) also changed at >3 wt% ash concentrations, with no apparent further change with increasing ash content. Key changes were not only an increased relative abundance of several potentially plant-growth-promoting rhizobacteria and fungi but also a decreased nematode abundance, suggesting that changes in the microbiome are likely a major factor for the substantial VAF effect observed in our experiment. We conclude that VAF with basaltic ash offers substantial potential benefits for agriculture as well as post-eruptive plant productivity on soils possibly associated with significant CO_2 sequestration from the atmosphere. However, VAF is a complex process that does not act by simple nutrient addition from its chemical inventory but rather acts by restructuring the soil growth environment.

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KEYWORDS
carbon cycle, geosphere–biosphere interaction, greenhouse growth experiments, plant–microbe–soil interaction, *Plectranthus scutellarioides*, soil fertility, soil microbiome, volcanic ash fertilization, volcano–ecosphere interaction

INTRODUCTION

Volcanoes conjure images of beautiful mountains that may unleash ferocious eruptions with catastrophic loss of life and property, but they are also known to produce fertile soils that nourish human populations. In fact, the fertility of many volcanic soils is legendary and led to suggestions to use volcanic ash as a fertilizer in agriculture (Ciriminna et al., 2022). However, the study of volcanic ash fertilization (VAF) remains at a rather early stage, even though early work suggests that it may have substantial potential, specifically for crop enhancements (Magnin et al., 2017; Rincón-Molina et al., 2022; Shoji & Takahashi, 2002; Zakharikhina et al., 2016) as well as for our understanding of planet Earth as a life-sustaining geochemical system in general. Increases in biological productivity from VAF offer a potential CO_2 sequestration mechanism that may be capable of removing some of the carbon that has been added to the atmosphere from volcanic outgassing. Increases in plant productivity from VAF may potentially also play a role in the geological nutrient cycle, replenishing soil nutrients that are lost from soils through weathering and erosion. However, all these potential global benefits of VAF remain in the realm of speculation until we better understand the processes that control it.

While the benefits of VAF to agriculture appear to be obvious (Ciriminna et al., 2022), the processes underlying its beneficial effects remain rather poorly understood for several reasons. First, the study of VAF is inherently interdisciplinary, requiring the complex integration of diverse research disciplines such as low and high temperature geochemistry, agronomy, soil sciences, ecology, botany, plant nutrition, or molecular biology, every one of them with distinct research methodologies and a largely non-overlapping scientific literature. Second, some of the key supporting arguments for volcanic soil fertility may not be as universally applicable as they seem at first glance. Certainly, there are particularly fertile soils in many volcanic regions, such as in Indonesia or in Southern Italy, but some volcanic soils in Japan, for example, are actually considered infertile (Dahlgren et al., 2004). A broadly accepted reason for the fertility of volcanic soils comes from the high potential nutrient content of volcanic rocks, leading to the promotion of volcanic ash as a fertilizer. Unlike other mineral fertilizers, however, volcanic ash is largely made of silicate glass and does not easily dissolve in water. Volcanic glass decomposes slowly, likely including complex microbe–glass interactions (Staudigel et al., 1995; Thorseth et al., 1992) and mostly transforming glass into relatively stable secondary phases such as palagonite, clays, or zeolites (e.g., Staudigel & Hart, 1983), rather than congruently dissolving into water as mineral fertilizers do. Hence, some of the ash's nutrients are slow and may be fixed into secondary phases, rendering them unavailable for plant growth. Lastly, the bulk of agricultural studies were carried out with the additional growth stimulation with mineral fertilizers (e.g., Xue et al., 2024; Zakharikhina et al., 2016), and it cannot be established how much of the observed growth increases are due to stimulation with mineral fertilizers and how much from ash addition alone. Our understanding of VAF is further complicated by the poor understanding of how the soil microbiome changes as ash is introduced to soil. Indeed, transformations in the soil microbiome are likely essential as microorganisms are directly responsible for nearly all biogeochemical transformations in soils and play a key role in nutrient dynamics (Nielsen et al., 2011; Sokol et al., 2022), silicate weathering and soil formation (Janssens et al., 2022; Vicca et al., 2022), and plant nutrient uptake (Berg et al., 2016). Lastly, microorganisms may also influence plant growth directly through the activity of plant-growth-promoting rhizobacteria (Backer et al., 2018) or indirectly by suppressing the activity of plant pathogens in soil that would otherwise inhibit plant growth.

Here, we have carried out a greenhouse experiment that illuminates VAF from a range of diverse, interdisciplinary perspectives, exploring potential plant growth behavior including the nutrient contents of leaf material, as well as the accompanying changes in soil microbiome.

EXPERIMENTAL AND ANALYTICAL APPROACHES

The goal of our VAF experiment is to explore how the addition of volcanic ash to soil may impact plant growth, nutrient uptake, and the soil microbiome in a controlled greenhouse setting. In such an experiment, it is important to choose experimental conditions and analytical

approaches that are realistic for VAF in natural and agricultural settings while providing reproducible and sensitive measurements including a statistically well-supported data analysis.

We chose the Coleus species *Plectranthus scutellarioides* as a model plant because it is likely well responsive to VAF as it is native to Indonesia where most soils frequently experience VAF. Its rapid growth and flowering behavior make it a particularly suitable model plant where VAF-related growth and reproductive behavior may be measured within reasonable growth periods.

We prepared a growth medium containing 50% unsterilized loamy mineral forest soil, 25% sphagnum peat, and 25% sterilized quartz sand. The peat was added to improve the water holding capacity of the substrate, while the sand and the peat addition together ensured limiting nutrient availability so that potential effects of the volcanic ash addition on nutrient supply would become optimally detectable. This growth medium is ash-free and hence has no pre-existing nutrients from volcanic ash that could be misidentified as being derived from the ash added. Ash-free soil also precludes that microbial populations are preconditioned to utilize ash as a fertilizer. This allows us to attribute changes in the microbial food web unambiguously to the addition of volcanic ash.

Basaltic volcanic ash was chosen as it is one of the most common volcanic rock types on earth, and it contains many potential nutrients that are suspected to be important to VAF. VAF involving silicic ash compositions is likely important as well, as they are produced by many explosive volcanoes, but exploration of this effect requires additional experiments. We also note that ash from the combustion of organic material may also play a role in soil fertility (Bang-Andreasen et al., 2020), but this is a process unrelated to VAF as it does not involve silicate glass, which is the main component of volcanic ash. The basaltic ash in our experiments was allowed to react with sulfuric acid prior to the experiment to simulate typical ash-acid reactions that occur in volcanic ash clouds. Completion of this reaction to near-neutral pH resulted in no significant pH changes in any experimental units after ash addition (tab. 2 at https://earthref.org/ERDA/2759/; Appendix S1: Figure S2).

Our 138-day experiment included 55 individual pots with clones of *P. scutellarioides*. They were prepared from cuttings from one mother plant, rooted, and grown in our non-volcanic substrate with various amounts of basaltic glassy ash added (Table 1). Five experimental units did not contain any ash (null references), and the remaining 50 units contained identical quantities of total growth substrate, with a range of ash concentrations in increments of 0.2 weight (wt)% up to 10 wt% (by mass), whereby the original contribution of ash-free soil decreased correspondingly.

TABLE 1 Chemical composition of the volcanic ash.

Component	Proportion (wt%)
SiO_2	45.84
TiO_2	2.18
Al_2O_3	12.57
FeO	12.87
MgO	7.85
MnO	0.24
CaO	12.86
Na_2O	2.84
K_2O	0.35
P_2O_5	0.18
Sum	97.78

Note: The ash was sampled from an active lava flow of Pu‘U O‘o Hawaii in 1994, quenched in fresh water and ground to <250 μm.

Gradient ash addition experiments outperform the traditionally replicated designs in detecting the underlying response shape and allow for inter- and extrapolation (Kreyling et al., 2018). We may also note here that the ash addition resembles conditions in nature or in agriculture, where any amount of ash added also reduces the amount of ash-free base soil by an equivalent amount.

We refrained from stimulating plant growth with traditional mineral fertilizers as it is typically done in agricultural practice (e.g., Zakharikhina et al., 2016). Traditional mineral fertilizers are designed to deliver the required nutrient in easily plant-accessible, typically water-soluble form. This type of fertilization, however, may also enhance the microbial-glass interactions (Staudigel et al., 1995; Thorseth et al., 1992) that may provide an additional boost to plant growth beyond what could be expected from VAF without mineral fertilizers.

At termination of the experiment, we photographed all individual pots (Appendix S1: Figure S1) and measured final vegetative height, cumulative inflorescence length, aboveground biomass production, and plant N uptake. We determined soil P, K, and N availabilities from a water extraction, electrical conductivity, and pH (Appendix S1: Figure S2; tab. 1 in https://earthref.org/ERDA/2759/). Aboveground biomass nutrient concentrations were measured in 11 aggregate samples of 5 individual plants each combined for ash free and 10 equally spaced aggregate samples between 0.2 and 10 wt% ash. Elemental abundances determined include macronutrients N, P, K, Ca, and Mg and micronutrients Na, Zn, Mn, Fe, Cu, and B (tab. 2 in https://earthref.org/ERDA/2759/).

We selected the best statistical model to describe and explore the response shapes of growth data and nutrient data based on corrected Akaike information criterion (AIC_c) (Hurvich & Tsai, 1989) and breakpoint analyses (Bai & Perron, 2003) (Figure 1; Appendix S1: Figures S2 and S3). We considered first- (linear) to third-order polynomials and then two models commonly used to describe ecological data, that is, the saturating Michaelis–Menten (Srinivasan, 2022) and the sigmoidal Gompertz function (Tjørve & Tjørve, 2017).

Soil microbiome profiles were obtained for soils from a representative set of 20 experimental units for the full ash concentration range (as indicated in Figure 1) through a genomic analysis of 16S rRNA and 18S rRNA gene amplicon markers by Illumina-MiSeq sequencing. They were processed using common bioinformatic workflows and tools, for example, the dada2 pipeline, crest4 database for taxonomic annotation, and FUNGuild classification for identification of functional guilds and trophic modes among Fungi (Callahan et al., 2016; Lanzén et al., 2012; Nguyen et al., 2016). Amplicon sequence variants (ASV) richness was analyzed with cor.test() from the "stats" R core package (R Core Team, 2024). NMDS ordinations were calculated for a matrix of Bray–Curtis dissimilarities in two dimensions for each dataset (16S rRNA and 18S rRNA). Ordination stress was calculated and found to be low (0.07 and 0.04 for 18S and 16S, respectively). Explained variance in the community compositions was analyzed with PERMANOVA using adonis2() from the "vegan" R package (Oksanen et al., 2016). Heat trees were made using the R package "metacoder" (Foster et al., 2017) summarized at class (16S rRNA) and phyla level (18S rRNA), showing only taxa with a summed abundance >0.1%. Node and branch width corresponded to mean relative abundance, the color gradient reflecting the log2-fold change in taxa abundance toward low (0%–2.6%, blue) or high (3.2%–9.8%, red) ash concentrations calculated using the R

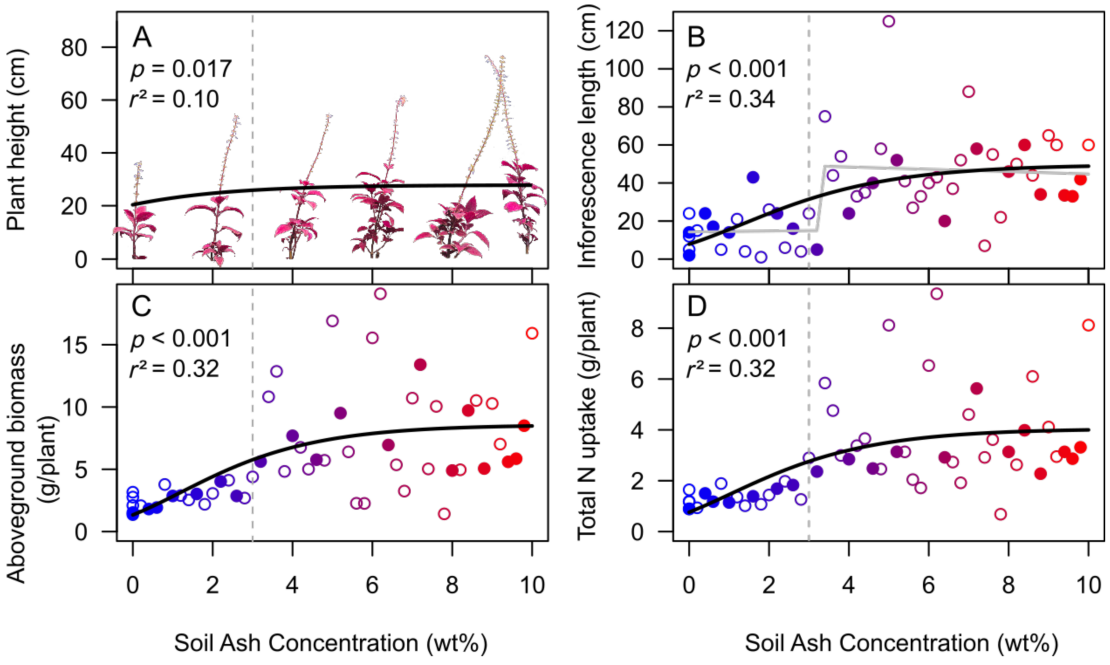

FIGURE 1 Plant growth in response to 0–10 wt% of volcanic ash fertilization (VAF) in non-volcanic soils. (A) Some representative plant images of *Plectranthus scutellarioides*, a coleus species, as a scaled visual indication of plant growth taken from the complete set of images in Appendix S1: Figure S1. The regression line displays the best-fitting model (see below) for height of the highest leaf. Individual symbols in (B) to (D) are color coded for ash content (low ash, blue; high ash, red) and samples with genomic data (filled symbols), representing an even spread along the ash concentration gradient. The data show increases in plant growth with increasing volcanic ash content in the soil, including reproductive effort measured as (B) cumulative inflorescence length, (C) aboveground biomass production, and (D) total N uptake, as proxy for plant nutrition (data from tab. 1 in https://earthref.org/ERDA/2759/). Sigmoidal Gompertz functions yielded best fit (based on corrected Akaike information criterion [AIC_c]) regressions in all cases (solid black lines); p values and r^2 values of these best fits are provided based on a linear regression between observed and fitted values. Breakpoint analyses found a significant breakpoint only for inflorescence length (gray line). The sigmoidal Gompertz functions yield a saturation of plant response at a 1.2-fold increase in plant height, a 3.7-fold increase in aboveground biomass production, a 4.1-fold increase in reproductive effort, and a 3.2-fold increase in total N uptake into plant shoots compared to our zero ash controls. The dashed vertical line at 3 wt% separates a low ash environment with minor growth and scatter from the high ash environment with much higher growth and higher variability.

package "matrixStats" (Bengtsson, 2009). Abundant taxa with a non-significant response are in gray (false discovery rate-corrected p values <0.05, Wilcoxon test). Raw sequencing files are available from NCBI (BioProject: PRJNA948353). The bioinformatic and statistical analysis script is available at Zenodo at https://zenodo.org/records/10984096.

More details regarding our experiment and the rationale for using materials and parameters, data acquisition, and data processing may be found in Appendix S1.

RESULTS

Plant performance and nutrition

Overall, VAF yielded a substantial increase in biological productivity as measured by aboveground biomass, reproductive effort, and nutrient uptake (Figure 1; Appendix S1: Figure S1). Response shapes along the ash addition gradient were not linear but best explained by sigmoid models (Gompertz equation yielding lowest AIC_c; Figure 1). The breakpoint analysis suggested a significant deviation from stability in the first-order polynomial at 3.2 wt% (95% CI: 2.0–3.4 wt%) ash addition for inflorescence length (Figure 1B). Generally, the low ash regime (below 3 wt%, dashed vertical line in Figure 1) showed a modest rise in aboveground biomass with increasing ash content (linear regression from 0 to 3 wt%, $R^2 = 0.41$, $p = 0.003$) and minor variability of growth data consistent with the variability of the five zero ash controls. At 3 wt% ash, variability and average growth increased dramatically, without significant further correlation between plant biomass production and ash contents ($R^2 = 0.0$, $p = 0.920$), supporting a threshold-like response around 3 wt%.

The Gompertz curve as best-explaining model suggested a 1.2-fold increase in plant height (Figure 1A), a 3.7-fold increase in aboveground biomass production, a 4.1-fold increase in reproductive effort, and a 3.2-fold increase in total N uptake into plant shoots at high VAF when compared to our zero ash controls (Figure 1B–D).

To gain a first-order understanding of the nutrient uptake during VAF, we determined the concentrations of a broad group of macro- (N, P, K, Ca, and Mg) and micro-nutrients (Na, Zn, Mn, Fe, Cu, and B) for 11 aggregate samples of plants grown in soils with 0–10 wt% ash (tab. 2 in https://earthref.org/ERDA/2759/). Plant nutrient concentrations may help us understand the processes of nutrient uptake, while the product of concentration and their total mass provide an estimate of the amount of nutrient taken up by the plant from the soil.

N and P concentrations in plants show a decrease, N by 10% (second-order polynomial, $p = 0.001$, $r^2 = 0.81$) and P by 40% (second-order polynomial, $p = 0.002$, $r^2 = 0.80$). However, this 10% reduction in N concentration contrasts with the overall threefold increase in total N extracted by the plants due to their large biomass increase (linear, $p > 0.001$, $r^2 = 0.79$; Appendix S1: Figure S3). The decrease in plant P concentrations also suggests that ash does not contribute to its uptake, and the substantial increases in biomass may deplete the P that is available from the soil, as total P taken up into plant biomass doubled along our VAF gradient (linear, $p < 0.001$, $r^2 = 0.76$; Appendix S1: Figure S3). Most notable may be an increase in Fe concentration by 32% (second-order polynomial, $p = 0.039$, $r^2 = 0.56$; Appendix S1: Figure S3). This increase in leaf Fe concentration can be compared to the high Fe content in the ash (Table 1), and hence, it is likely that this increase is a direct function of VAF.

The total nutrient uptake, the product of leaf concentration and biomass, shows a massive increase for all nutrients mostly due to the large variation in biomass that effectively masks the relatively small and complex variation in plant nutrient concentrations. Hence, consistent with the dramatic increase in biomass, the total nutrient uptake also shows a steep trend toward a threefold increase at high ash concentrations, providing an additional boost to the likely impact of VAF on crop yields and natural bioproductivity (Figure 1; Appendix S1: Figure S3).

Soil microbiome restructuring

Two remarkably distinct community clusters in both the eukaryotic and prokaryotic microbiomes were revealed by the ordination analyses (Figure 2), separated into a low and high ash addition group (blue colors, 0–3 wt% ash and red colors, 3–10 wt% ash addition), which could explain 36.5% and 27.5% of the community variance of Pro- and Eukaryota, respectively (PERMANOVA, $p < 0.05$; Appendix S1: Table S1). The tight clustering of the two groups highlights the high internal compositional similarity within the two microbiome clusters, without any gradual transition trend between them. That suggests that no major change in microbiome composition occurred before 3 wt% ash addition, whereas higher concentrations led to dramatic changes in the microbiome, coinciding with the increased plant growth. Furthermore, the changes in microbiome composition at 3 wt% ash were also reflected in the amount of total DNA extracted from the soil-ash substrates, which showed a similar two-state system with high amounts of DNA extracted from samples with up to 3 wt% ash and 50% reduction above 3 wt% ash (with no overlap between the two growth regimes above

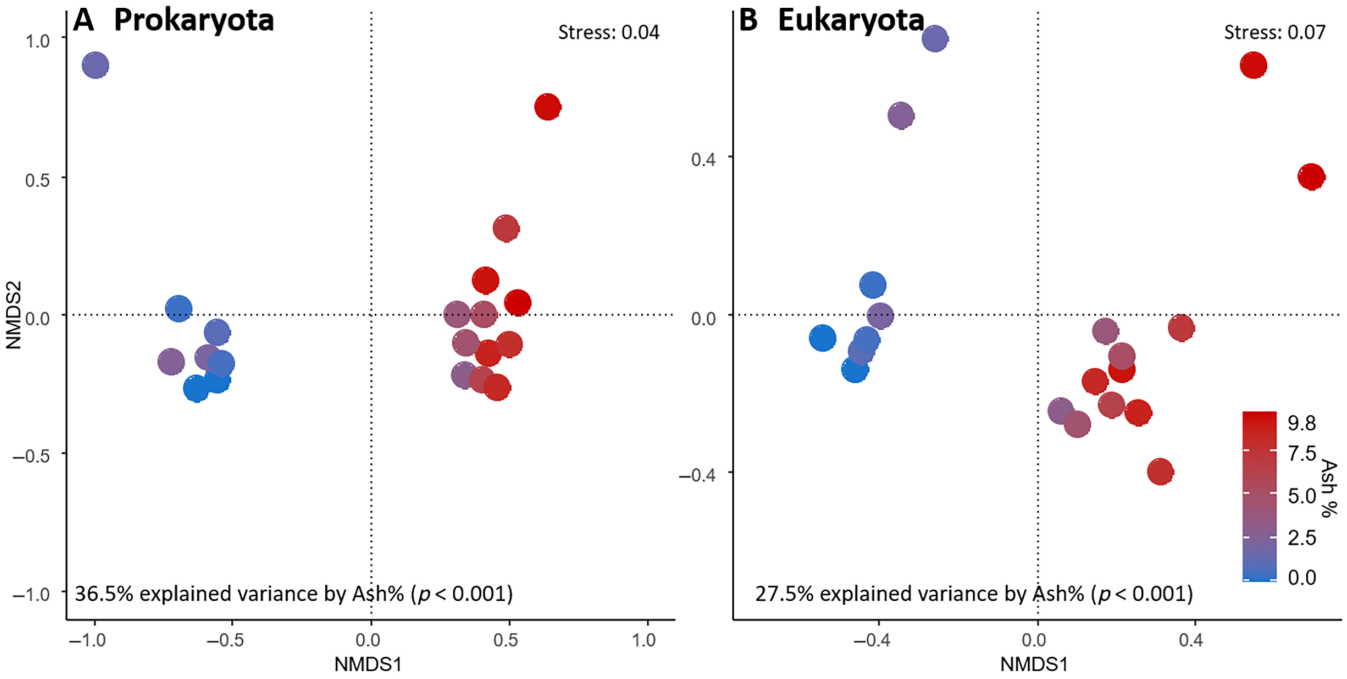

FIGURE 2 Microbiome composition affected by volcanic ash fertilization (VAF). Non-metric multi-dimensional scaling (NMDS) ordinations were generated for the two microbiome profiles of (A) Bacteria/Archaea and (B) Eukaryota including the first and second NMDS axes. Stress values were 0.04 and 0.07 for the (A) 16S rRNA genes (in total 8453 ASVs) and (B) 18S rRNA genes (3830 ASVs), respectively, indicating a good representation of the data (dis)similarity between samples in two-dimensional space (≤0.05 is considered a very good fit; Clarke & Ainsworth, 1993); for details, see Appendix S1. Samples are colored similarly to the symbols in Figure 1 (marked with filled symbols). Ash% was found to explain 36.5% and 27.5% of the community variance for Pro- and Eukaryota, respectively (PERMANOVA, $p < 0.05$; Appendix S1: Table S1). Data fall into two distinct clusters for the low ash and high ash substrates (<3 > wt%), and there is no indication of any gradual change in microbial composition between them, and when tested, a separation into these two groups explained 46.6% and 29.0% of the community variance for Pro- and Eukaryota, respectively. This suggests a dramatic shift in the microbiome that is likely to commence at ash contents higher than 3 wt% and does not change as ash content increases to 10 wt%. The data in the low ash substrates are in the same cluster as the ash-free substrate, suggesting that the low ash microbiomes are statistically identical to an ash-free substrate, but they are very different from the high ash group.

and below 3 wt%; Appendix S1: Figure S4). No relationship between ash addition and ASV richness was observed for bacteria ($p = 0.37$), but a tendency toward a decline in Eukaryotic ASV richness with increasing ash addition was seen ($p = 0.07$; Appendix S1: Figure S5).

Which microbiome members responded to VAF? A large number of Bacteria and Eukaryota showed significant changes in their relative abundance between high (>3 wt% ash addition) and low (<3 wt%) ash addition illustrated in a heat-tree representation in Figure 3. Here, the width of nodes and branches offers a measure of their abundance in ash-rich (red) and ash-poor experiments (blue). The higher order taxa that were consistently more abundant with high VAF included the Firmicutes (especially Bacilli), Actinobacteria (class), Gemmatimonadetes, Parcubacteria, and Cytophagia (Figure 3A), while Alpha- and Gammaproteobacteria, Acidobacteria-GP6, Acidimicrobiia, and some classes of Bacteroidetes were less abundant at high ash concentrations. Among the Eukaryota, it was remarkable that most fungi (the large phyla Ascomycota and Basidiomycota) had a reduced relative abundance, only with the exception of members of Chytridiomycota, which were relatively more abundant in the high-ash group. The most abundant animal group (Nematoda) and some "protists" (Tubulinea and Apicomplexa) were also negatively impacted by ash addition. By contrast, cercozoan "protists," Euglenozoa, and Labyrinthulomycetes showed an increased relative abundance (Figure 3B). Thaumarchaeota, the dominant archaeal group, did not show a response to ash addition (not shown in Figure 3).

DISCUSSION

Plant performance and nutrition

VAF with basaltic ash in our soil substrate had a pronounced but complex impact with a roughly threefold increase in primary production, nutrient uptake, and

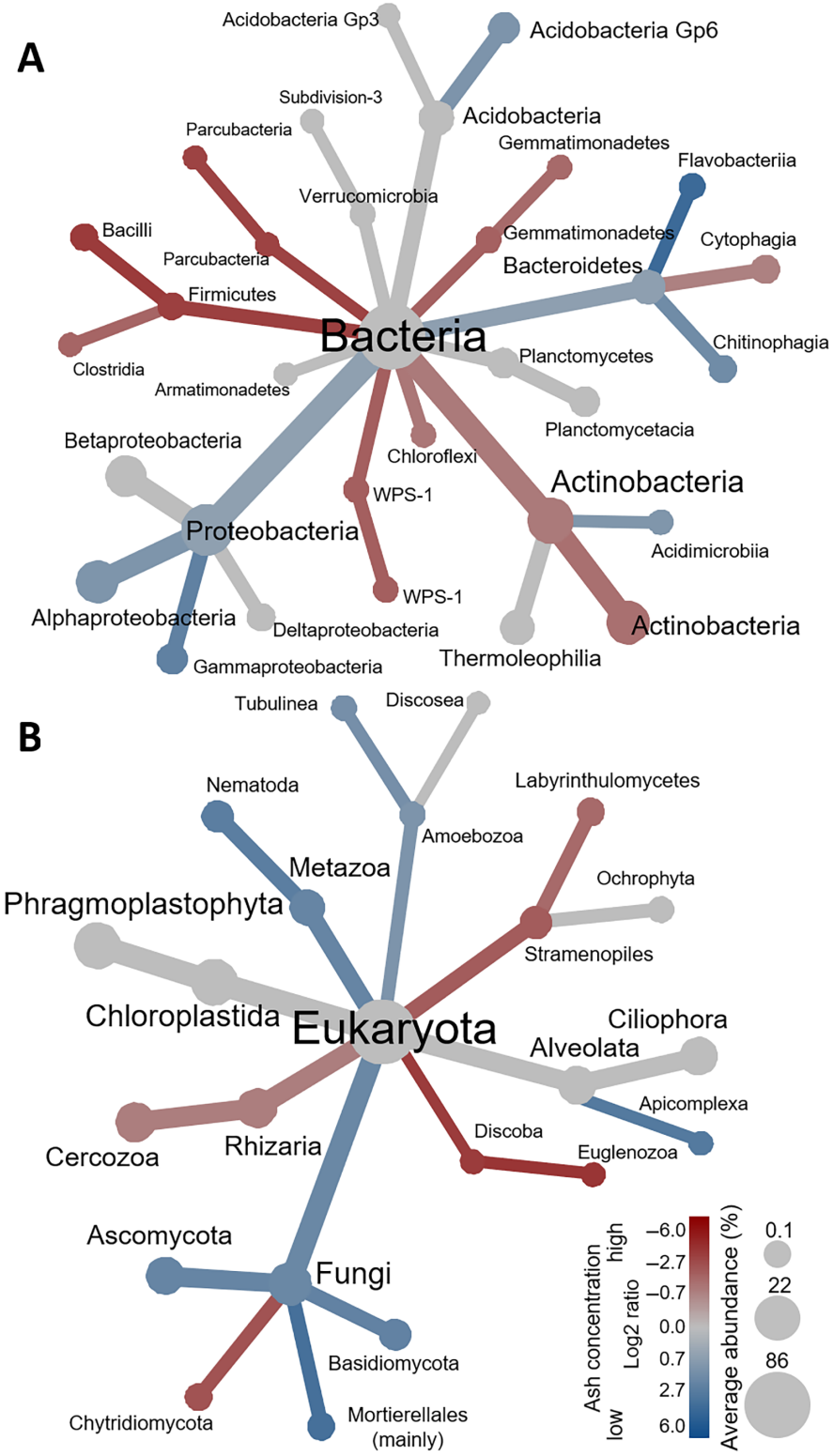

FIGURE 3 Volcanic ash fertilization (VAF)-responsive taxa. Taxa (>0.1% relative abundance) summarized at class and phyla level for (A) Bacteria and (B) Eukaryota, respectively. Archaea not shown. Node and branch width indicate the relative abundance of a given taxa. Color indicates predominance of the taxa in low ash content substrates (<3 wt%, blue) or high ash content substrates (>3 wt%, red), and node branches with non-significant changes between low and high ash concentrations are denoted in gray (false discovery rate corrected $p < 0.05$, Wilcoxon tests). Branches with insignificant changes (gray) are rare, suggesting that the microbiome for ash rich and ash poor substrates are reordered at high taxonomic resolution (phyla and class level) and suggests substantial changes in the soil food web structure in response to the quantity of ash addition. These changes are particularly well developed in phyla that are expected to be relevant for nutrient cycling or suppression of pathogens in soils (see main text).

reproductive effort of *P. scutellarioides*. Such a substantial increase in our model plant productivity supports the suggestions that volcanic ash may be used as an agricultural fertilizer (e.g., Ciriminna et al., 2022; Xue et al., 2024). Furthermore, it supports the contention that VAF may have the potential to remove CO_2 from the atmosphere on a local or global scale, over time scales that may vary from post-eruptive to geological time periods. Hence, we speculate here that geological carbon cycles (Marty & Tolstikhin, 1998) may have to consider not only volcanic emissions of CO_2 but also the sequestration of CO_2 due to increased post-eruptive biological sequestration.

The observation of two distinct growth regimes below and above 3 wt% suggests that VAF likely involves at least two processes that differ between the low-ash and high-ash regimes. The weak linear increase in plant performance in the low-ash regime may be attributed to a number of processes, possibly including catalytic processes (Zakharikhina et al., 2016), cation exchange (CEC) effects (Kowalenko & Yu, 1996), microbially mediated silicate weathering of ash (Vicca et al., 2022), and/or a direct contribution from salts that have been produced during the leaching of the ash prior to the experiment. All these processes would deliver gradual growth effects that are consistent with the gradual addition of ash in the low-ash regime, but our data do not allow raising any of them above the level of speculation. None of them significantly elevated plant productivity.

VAF with more than 3 wt% ash, however, involved a strong increase in plant performance, and its growth behavior reveals important details about processes of VAF. The lack of a clear progressive plant growth response at high ash content and its high variance suggests a complex systemic response to the ash addition at these levels, which likely goes beyond simple fertilization. Such a non-linear, non-progressive response to ash addition is also consistent with a data set provided by Xue et al. (2024), who observed an increase of ca. 13% in melon yield compared to the 0% control when adding 5%, 15%, or 25% volcanic ash, whereby yield decreased at higher ash concentrations, 35% and 45%. These non-linearities point toward a process that is not progressively dependent on ash concentration but largely depends on the establishment of a most beneficial range of ash concentrations that requires a minimum amount of ash addition and likely ends when ash becomes too abundant. Our nutrient data largely mirror the growth data, but they also independently add to our understanding of VAF processes, in particular, the data for Fe and N. Fe is an important often limiting micronutrient, and the observed increase in Fe contents and concentrations with ash content may be related to its high abundance in ash, mostly in its bioavailable form Fe^{2+} (FeO = 12.9 wt%; Table 1). Hence, we might speculate that Fe^{2+} from volcanic ash may have directly or at least indirectly affected plant nutrition (Schmidt et al., 2019). Here, it is important to note that the Fe content in ash is very high when compared to the nutritional requirement of Fe as a micronutrient. Hence, dissolution of extremely small quantities of ash might already suffice to make a significant impact on plant growth. By contrast, there is no evidence for ash-related direct fertilization from the dramatic increase in N uptake by plant matter from VAF. Ash does not contain N, rendering the soil organic matter, the atmosphere, or the turnover in microbial biomass as potential sources for the N additionally taken up into the plants with VAF. N is known to be the major limiting factor for coleus plant growth (Chen et al., 2017), and growth should be particularly limited without an N source in the ash augmentation. This strongly suggests that ash addition only indirectly increases the N uptake likely by involving a complex ecosphere response that likely includes some level of microbial mediation.

Soil microbiome restructuring

The microbiome data (Figures 2 and 3) indicated a major community adjustment to the addition of basaltic volcanic ash to forest soil as the ash content exceeded 3 wt%. This adjustment was correlated with the major plant growth increase and possibly is an integral part of a remodeled microbial food web within new nutrient acquisition pathways (e.g., Mehlferber et al., 2022). These pathways likely involved the indirect utilization of volcanic ash for N acquisition as well as the potential growth-promoting role of Fe. While these correlations (plant and microbial responses) were quite apparent, they are difficult to interpret in terms of specific microbial functions due to the highly diverse metabolic capabilities of the taxa identified as well as the interactions among microorganisms themselves and with ash. Future RNA studies, however, may offer a particularly promising research project to help with these interpretations.

Among Bacteria, the strongest relative increase in abundance was found in Firmicutes, especially the Bacilli, and in phylotypes of the family Bacillace. Many species of Bacilli are known for being plant-growth-promoting rhizobacteria (PGPR; Backer et al., 2018) such as *Bacillus subtilis* and *Bacillus velezensis* (Niu et al., 2020), and thus, members of this class may have played a direct role in the positive plant response. Likewise, *B. velezensis* has been shown to suppress the eukaryotic plant pathogen *Phytophtora sojae* (Oomycota, in soy bean via bacilysin production; Han et al., 2021),

thus representing an example of how cross-domain inter-species interactions might also affect plant growth, in this case indirectly through the suppression of plant pathogens.

Key changes among the Eukaryota include, in particular, the order Tylenchida, one of the two most abundant groups of Nematoda, which was significantly reduced (2.1-fold change, $p = 0.042$) under high ash conditions. These are known as plant-parasitic nematodes (NEMAPLEX, 1999 onward) and such pest release likely also contributed to the positive plant growth response. Whether this was a direct effect of the ash addition or secondarily via biotic interactions with, for example, plant roots or other microorganisms cannot be discerned from our data set. Exploring potential changes of functional classes of fungi through a FUNGuild analysis (Nguyen et al., 2016) revealed only inconspicuous changes among functional guilds (Wilcoxon test; Appendix S1: Table S2). Similarly, we did not identify a response in the arbuscular mycorrhizal forming fungal class Glomeromycetes (which in general had a very low relative abundance, ~1.1% of all fungi).

But what about N provision to *P. scutellarioides*? Microbially mediated fixation of atmospheric nitrogen may have played a role, but our data neither support nor reject this hypothesis. However, in addition to taxonomic changes in the microbiome that may impact the availability of N in soils, some information may be gleaned from the total yields of extractable DNA that were substantially lower (per gram dry soil) in the high ash group (Appendix S1: Figure S3). Such a change in DNA yield could indicate a higher death rate/turnover within the microbiome or, at least, that the transition from one species composition to the other must have resulted in an increased turnover of microbial biomass. Both processes would result in microbial necromass, which might have acted as a plant growth driver providing a source of organic and inorganic N in the soil (Camenzind et al., 2023; Sokol et al., 2022). Whether this was a transient event (going from one state to the other) or a permanent change in N acquisition pathways (increased turnover) is an important question for future experiments.

Implications for natural settings

VAF effects in nature may vary for different ash compositions, climatic conditions, plant species, soil types, pre-existing microbiomes, or timing of eruption and post-depositional temporal scales. Bearing these complexities in mind, we focus on three first-order issues: the composition of ash in VAF, its dispersal into soils, and the timing of VAF in nature and in our experiment.

The choice of ash composition in our experiments may have an important impact on the type and quantity of nutrient addition to soils. We used a mafic (Mg- and Fe-rich) ash because it is the most common volcanic rock type on earth that is likely to have a major impact on the nutrient cycle, even though ash deposits with global reach tend to be more silicic. Such a more global distribution of basaltic ash, however, is not unheard of, as it may be supported by observations of ash clouds from the predominantly basaltic Icelandic volcanoes (e.g., 2010 Eyjafjallajökull eruption) that reached well beyond Iceland, well into Great Britain and the rest of Europe, causing significant disturbances to air traffic. The widespread distribution of these ash clouds allows us to infer that at least some of this basaltic ash has been deposited over much of Europe. Furthermore, mafic ash is often considered particularly "fertile" because the nutrients it carries are in basaltic glass that tends to dissolve more rapidly than silicic glasses (Wolff-Boenisch et al., 2004), enhancing the potential impact of basaltic VAF. Irrespective of the ash composition, however, almost any ash may contain some nutrients that have been brought up to the surface from earth's interior and therefore contribute to soil fertility and the nutrient cycle in soils. Our study of VAF via basaltic ash is an important step forward, but similar studies remain to be done for a range of ash compositions for a systematic and quantitative evaluation of the global impact of VAF.

Ash distribution in our experiment differs from natural ash falls. We mixed the ash into the soil substrate, creating a homogeneous mixture. This assured that all roots had instant access to volcanic ash, providing a reproducible experimental setup and timeline between ash addition and subsequent plant growth. In nature, however, ash is top-loaded onto the soil, and once deposited, it may remain in place and/or filter into the soil by bioturbation or as it is imbibed by rain as a function of soil porosity and water repellency (e.g., Saputra et al., 2023). Alternatively, ash may be redeposited by wind or rain-runoff before it has the opportunity to mix and interact with the local soil, resulting in spatially uneven VAF effects.

Predicting the growth stimulation of a given ash fall is essential for an estimate of the regional or global increase in biomass production, but it is highly complex due to the spatio-temporal heterogeneity in soil ash concentrations combined with its non-linear effects on plant growth. Still, our data allow us to consider the potential maximum fertility impact of an ash fall with the basaltic ash we used in our experiments. Based on 3 wt% of ash as the threshold for maximum VAF, a 3-mm ash layer

could potentially fertilize a 10-cm soil layer. The upper 10 cm of the soil contains 44% of all roots in grasses and 25% of trees, respectively (Jackson et al., 1996), as well as the lion's share of the microorganisms (Murphy et al., 1998). While such considerations may have little predictive power for the actual VAF-generated increase in bioproductivity, they provide an upper bound for how much primary production could be enhanced by a particular ash fall.

Lastly, VAF also critically depends on the type and timing of post-eruptive processes that facilitate nutrient release for time periods much longer than our four-month experiments. One key control comes from the ash dissolution kinetics (Wolff-Boenisch et al., 2004), but grain size is likely to play a big role in determining the total chemical extraction from dissolution as well. Our relatively fine-grained (silt-sized) glass, for example, is representative of ash that is deposited distant from a volcano, and it is likely to dissolve more effectively than coarser grained deposits with a lower specific surface area deposited closer to a volcano. Besides dissolution, nutrient release from ash may further be influenced by temporal changes in microbial communities such as the one observed here, and the development of biofilms as well as a number of soil-hydrological and climatic variations that by themselves will determine the microbial communities as well. Our four-month experiment showed a complex but effective set of conditions yielding a large VAF growth response, but VAF is probably longer lived and may continue for years after deposition, with an enhanced growth of biomass and potentially also CO_2 sequestration that may remove some of the carbon that has been added to the atmosphere from volcanic outgassing. Current models of the geological carbon cycle (e.g., Marty & Tolstikhin, 1998) do not consider such a potential flux, and it is important to better constrain these types of potential fluxes so we may include them if they are significant.

CONCLUSIONS

Volcanoes are best known for their violent eruptions and destructive potential, but they are also widely credited with the benefit of fertilizing soils, yet the processes of volcanic fertilization remain poorly understood. Our greenhouse VAF experiments using basaltic ash and the Coleus species *P. scutellarioides* as a model plant yielded some important insights into these processes:

1. VAF offers an effective, but non-linear boost in plant growth, with very little effect at low ash contents (<3 wt% ash) but a more than threefold increase in aboveground biomass production, plant reproductive effort, and soil nutrient extraction at higher ash concentrations. This underscores the potential for VAF increasing agricultural yields as well as supporting the enhanced bioproductivity in non-anthropogenic soils and atmospheric CO_2 sequestration.
2. VAF at high plant growth rates above 3 wt% ash involves a substantial restructuring of the soil microbiome, including the increases in potential plant-growth-promoting rhizobacteria but also indirect growth support through the reduction of plant pathogens such as nematoda.
3. VAF must not be understood as a simple, direct nutrient addition process but rather as an indirect process of fertilization that is based on complex restructuring of the soil chemical and microbial environment that results in enhanced nutrient uptake from the non-volcanic portion of the soil (e.g., N) as well as the ash itself (likely Fe).

AUTHOR CONTRIBUTIONS

Hubert Staudigel: Conceptualization; experimental design; ash production; interpretation; writing: original draft; review and editing; resources. **Mathilde Borg Dahl:** Investigation; formal analysis of genetic data and interpretation. **Yohannes B. Tesfay:** Investigation; data curation; experiment; data collection. **Ilka Beil:** Investigation experiment and pretrials. **Tim Urich:** Investigation; resources; microbiological analytics; interpretation. **Juergen Kreyling:** Conceptualization; methodology; resources; validation; investigation; experimental design; plant data analysis; interpretation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data are included in this paper or available on EarthRef.org Digital Archive; growth and nutrient data: https://earthref.org/ERDA/2759/. Code (Borg Dahl, 2024) is available from Zenodo: https://zenodo.org/records/10984096. Molecular data are available from the National Center for Biotechnology Information (NCBI): BioProject PRJNA948353.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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