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Full Length Article

Revealing Rhizosphere of Edelweiss (*Anaphalis longifolia*), Plant Pioneer Species in the Volcanic Mountain Ecosystem in Indonesia

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Abstract

Edelweiss (*Anaphalis longifolia* (Blume) Blume ex DC.) is a pioneer plant on young volcanic soil rich in sulfur (S), developing a mono-specific forb layer. Unfortunately, their population is threatened by illegal over-harvesting. Hitherto, the autecological understanding of its rhizosphere is limited. The study aimed to observe compounds and functional microbial populations that may play important roles in this challenging environment. The study was conducted in the Kelimutu National Park (Flores, Indonesia), between 1200 and 1600 meters above sea level. Soil samples were collected from both bulk soil and root zone. Chemical analysis for soil and plant tissue samples used gas chromatography and mass spectrometry (GCMS). Soil microbial populations were analyzed using plate count and most probable number (MPN) methods. Data revealed that the most abundant root exudates were organic acids, which contribute to the formation of rhizosphere community. Colonization of functional groups is found to be more strongly determined by the rhizosphere assemblage than by elevation. Several substances recognized as phytotoxins may play an important role against pests and diseases in the soil. Data further revealed that rhizosphere assemblage by edelweiss produces substances classified as allelochemical compounds assumedly suppressing other plants. Compounds classified as insect repellents were also found in the leaf tissue. It can be concluded that the success of edelweiss as a pioneer in mono-specific stands on the volcanic soil of high altitude can be attributed to its rhizosphere assisting in reducing availability of S and improving C, K and N. © 2024 Friends Science Publishers

Keywords: *Azospirillum thiophylum*; Functional microbes; Mountain savannah; Pioneer plant; Volcanic soil

Introduction

Edelweiss (*Anaphalis longifolia* (Blume) Blume ex DC.) is a light-loving pioneer plant and survives in barren soil and cool air on young volcanic soil in mountain forests (Taufiq 2009). The plant is adapted to develop as a savannah mono species (Utomo 2017) at 1600–3600 m above sea level (masl) and can be observed in mountain forests on the border between the forest and open areas (Van Steenis *et al*. 2006).

Edelweiss belongs to the Asteraceae family, which has golden-colored flowers with a flat flower base. This plant is a shrub reaching a height of 4–8 m, with linear-shaped leaves (the length is equal to ten times the width), pointed, bearing white hairs like wool, and is 4–6 cm in length, and 0.5 cm wide (Van Steenis *et al*. 2006). Edelweiss is characterized by stalks, leaves, and flowers covered with a layer of white fibres as a form of adaptation to the climate at extreme altitudes. Other edelweiss adaptations are shape and color, which depend on the environment in which they grow

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(Van Steenis *et al*. 2006). Globally, it is estimated that there are approximately 110 edelweiss species (*Anaphalis* spp.), their distribution is mainly in Southeast and South Asia (Taufiq 2009). One species is found in North America, popularly known as the western pearly everlasting (*Anaphalis margaritacea*), while the species distributed in the alpine mountain region are of the genus *Leontopodium* (Van Steenis *et al*. 2006). Several species of *Anaphalis* exist in Indonesia, including *A. javanica*, *A. longifolia*, *A. viscida*, and *A. maxima* (Van Steenis *et al.* 2006). The Javanese edelweiss (*A. javanica*) is the most dominant species, while the least hardy species is *A. maxima* (Kurniawan *et al*. 2014). Recently, this endemic protected species has been categorized as threatened and included in the International Union for Conservation of Nature (IUCN) Red List. They have been experiencing a drastic population decline, leading to critical status or CR (Taufiq 2009).

Every plant has a mechanism regulated by the rhizosphere for adaptation and survival (Widyati 2018). Plants release compounds from their roots that encourage beneficial bacteria to flourish in the rhizosphere (Bais *et al.* 2006; Compant *et al.* 2010). Many studies showed that plant-microbial relationships are often species-specific (Berg and Smalla 2009). Varying root exudation and rhizodeposition lead to plant species composition designated by the functional and taxonomic groups in the rhizosphere (Ciccazzo *et al.* 2014). According to research conducted on monoculture plants, plant species affect specific bacterial populations (Smalla *et al.* 2001; Kowalchuk *et al*. 2002). Therefore, plants can modify their rhizosphere microbial community in a host-dependent way. As a result, universal inferences concerning plant species-specific connections, predominantly the structure and composition of soil microbial society, cannot be drawn (Ehrenfeld *et al.* 2005).

A plant species favors a distinct microbial community in the rhizosphere (Haichar *et al*. 2008; Turner *et al*. 2013). Furthermore, the location may also affect soil microbial structure, particularly if microbial groups have limited dispersal, leading to site-specificity of microbial communities (Ettema and Wardle 2002; Fierer and Ladau 2012). Previous studies have suggested that the distribution boundaries of microorganisms are not significantly determined. They can exist in all sites, but the environment specificity shapes the microorganism communities (O'Malley 2007).

Alpine ecosystems have attracted considerable attention in worldwide dynamics, mainly because of their role in global carbon storage (Wu *et al.* 2017a; Zhang *et al*. 2017). In mountain habitats, elevation gradients and varied precipitation regimes affect vegetation composition and soil characteristics, which influence the structure of the resulting microbial population (Lazzaro *et al*. 2015). Mountain habitats are determined by altitude levels and severe climatic changes within narrow geographic coverage, making these landscapes suitable for natural laboratories (Körner 2007); most studies report that altitude-related environmental conditions strongly affect the rhizosphere assemblage (Donhauser and Frey 2018).

Dinitrogen fixation, either by non-symbiotic or symbiotic bacteria in the root system, is important in environments such as volcanic ash where nitrogen is lacking while other nutrients are available (Ishaq *et al*. 2020). Volcanoes with less frequent eruptions still pose challenging conditions for plant growth, where fumaroles cause high atmospheric SO_2 concentrations (Carn *et al.* 2017). Active volcanoes form a challenging environment for plant growth despite the high nutrient availability of volcanic ash once it starts to weather (Fiantis *et al.* 2010). Soil microbial and rhizosphere processes also control soil formation and terrestrial carbon storage (Wu *et al.* 2017b; Zhang *et al.* 2017).

Edelweiss is Indonesia's most well-known pioneer plant in mountainous volcanic areas (Van Steenis *et al.* 2006). Pioneer plants modify the aboveground microclimate, allowing other plants to get established and take over (Holle and Tsuyuzaki 2018). They can also modify soil conditions by effects on the rhizosphere and impacts of aboveground litter. To date, information on the performance of root exudates and their soil communities for edelweiss plant species specificity is insufficient. As a result, there is no reference for the function of the edelweiss rhizosphere in regulating the adaptive mechanisms at high-volcanic mountain habitats.

In view of the above information, this study was aimed at to observe the adaptation strategies of the edelweiss rhizosphere to maintain the population size in the wild as a basis for *ex situ* conservation to increase the population size of this species. We hypothesized that edelweiss shape rhizosphere to conquer new habitat by secreting root exudates to invite and maintain the structure and composition of their soil communities. In addition, rhizosphere control in producing allelopathic compounds to repel surrounding plants, hence creating mono savannah. The rhizosphere also controls pest repellant production of above ground tissues. Therefore, the objectives of study were to: 1) observe content and types of root exudates in edelweiss rhizosphere in various elevation; 2) observe functional groups of soil microbes in edelweiss rhizosphere in various elevation and 3) analyze the secondary metabolites both in edelweiss rhizosphere and above ground tissues in various elevation.

Materials and Methods

The study was conducted at the Kelimutu Lake National Park on Ende Island, East Nusa Tenggara, Indonesia (Fig. 1), at altitudes of 1247, 1321, and 1585 m above sea level and coordinates of 8°43'–8º48'N and 121°44'–121º51'E. The sites were divided into three elevations: low, intermediate, and high (Fig. 1). The samples of rhizosphere soil, bulk soil, and plant tissues were analyzed in the laboratory. Chemical characteristics were analyzed using the gas chromatography and mass spectrometry (GCMS) method, and the soil microbial population was analyzed using the plate count and most probable number methods. Data on crucial environmental conditions such as temperature and altitude were also collected.

Material sampling

Soil and plant tissue samples were collected on two days in March 2021 at three sites along the western slope of Mount Kelimutu, ranging from 1200 to 1600 masl, with 150–200 m intervals. At each altitude, five composite soil (0–20 cm depth) samples (approximately 1000 g) were collected from three points around three plants, i.e., 15 rhizosphere soil samples were collected. This on-site composition made it easier to transport samples because of the high and hard mountainous terrain that can only be reached by walking. Fifteen bags of rhizosphere soil samples adjacent to the plants were collected by removing every piece of root to ensure root-free samples. A similar sampling method was conducted on the non-rhizosphere (bulk soil) samples as a control treatment. Five hundred grams of shoots (leaves and twigs) were collected from five sample composited plants at each altitude to analyze chemical compound configuration in plant tissues. The samples of soil (n=30) and plant (n=15) were immediately transported to the laboratory in the cooler boxes for further analysis.

Chemical analysis

The rhizosphere soil, the bulk soil, and leaf samples were analyzed using the GCMS method to determine the concentration of compounds categorized as sugars, organic acids, allelochemicals, organic compounds, growth regulators (auxins, cytokinins, and gibberellins), as well as principal soil enzymes such as phosphatase, nitrogenase, and cellulase, etc. In addition, general soil fertility analysis was also conducted on rhizosphere and bulk soils, i.e., soil pH, content of organic carbon (OC), total nitrogen (N), available phosphorus (P), available potassium (K) and available sulfur (S), because volcanic soils comprise the specific habitat of edelweiss using routine procedures.

Soil microbe isolation

Before isolation, the rhizosphere soil was progressively dissolved in a physiological salt solution (0.85% NaCl) until the seventh dilution series was reached. Briefly, 100 g of rhizosphere soil was added to a solution of 1000 mL of 0.85% NaCl and thoroughly shaken (10^{-1} dilution series). From this solution, 1 mL was taken, added into a 10 mL 0.85% NaCl solution and thoroughly shaken (dilution series 10^{-2}). This was done successively up to six times until a solution of 10^{-7} was obtained. Furthermore, isolation and estimation of the functional rhizosphere microbial population were carried out as follows.

a. Cellulolytic microbial groups were collected using a solution of 10^{-5}–10^{-7} dilution and then isolated by the plate count method using carboxymethyl cellulose agar.
b. The free N_2-fixing microbial group was collected using a solution of 10^{-3}–10^{-5} dilution and then isolated using the most probable number (MPN) technique with Azospririllum medium.
c. The phosphate-solubilizing microbial group was collected from a solution of 10^{-4}–10^{-6} and then isolated by the plate count method using Pikovskaya agar media.

Data analysis

The effect of the altitude, a fraction (rhizosphere and bulk soil), and the interaction on microbial population was analyzed using a two-way analysis of variance (ANOVA) of JMP 14 Software (Sall *et al.* 2005).

Results

The study finds that 370 compounds were isolated from the rhizosphere soil. It can be further classified into five functions (Table 1). The most found compounds were antimicrobial (organic compounds, volatile organic compounds, and various fatty acids), energy sources (alcohols and amino acids), nutrient acquisition and community shape (organic acids), stress responses, induction of system resistance (volatile organic compounds), and allelochemicals (steroid alkaloids, terpenes, terpenoids, and aldehydes). However, in the bulk soil, without the influence of root activity, only 71 compounds were found, almost all of which were antimicrobial (Table 1). The composition of functional rhizosphere components (Fig. 2) shows that more variety and functions of root exudates were found in the soil root zone (Fig. 2a) compared to the bulk soil (Fig. 2b). The rhizosphere of edelweiss secreted about 44% of compounds functioning as nutrient acquisition and community shaping, 24% antimicrobial, 18% energy sources, 8% plant response and defence systems, and 6% allelochemicals. The bulk soil contains 90% antimicrobial compounds, 7% nutrient acquisition and community shape, and 2% energy sources. No defence system and allelochemical compounds are found in the bulk soil.

Fig. 3 shows that the formation of the rhizosphere attracts the colonization of the main functional groups, namely cellulose degraders, nitrogen fixers, and phosphate solubilizers. Regardless of the type, they were classified as functional groups, critical in supplying nutrients to plants. The nitrogen-fixing bacteria (*Azospirillum* sp.) group was further characterized, with those from the rhizosphere region exhibiting characteristics similar to *Azospirillum thiophyllum* (Reis *et al.* 2015). In addition to fixing nitrogen, the Azospirillum group uses the process of assimilation of element S to obtain energy. Table 3 shows a very poor population of nitrogen fixers and phosphate solubilizers in the bulk soil.

Table 1: Summarized classification of root exudate analyzed based on its major functions

Altitude	Number of identified compounds	Role in the rhizosphere				
		Antimicrobial	Energy source	Nutrient acquisition & community shape	Stress respond & resistance system	Allelochemical
T1RS	163	33	35	69	12	14
T2RS	116	29	15	58	9	5
T3RS	91	27	16	36	10	2
TOTAL	370	89	66	163	31	21
Average	123.33	29.67	22	54.33	10.33	7
T1 BS	24	19	1	2	-	-
T2BS	25	20	-	1	-	1
T3BS	22	23	-	2	-	-
TOTAL	71	62	1	5	-	1
Average	23.67	20.33	0.33	1.67	-	0.33
RS: BS ratio	5.21	1.50	66.67	32.53	emerge	Emerge

RS: rhizosphere soil; BS: bulk soil

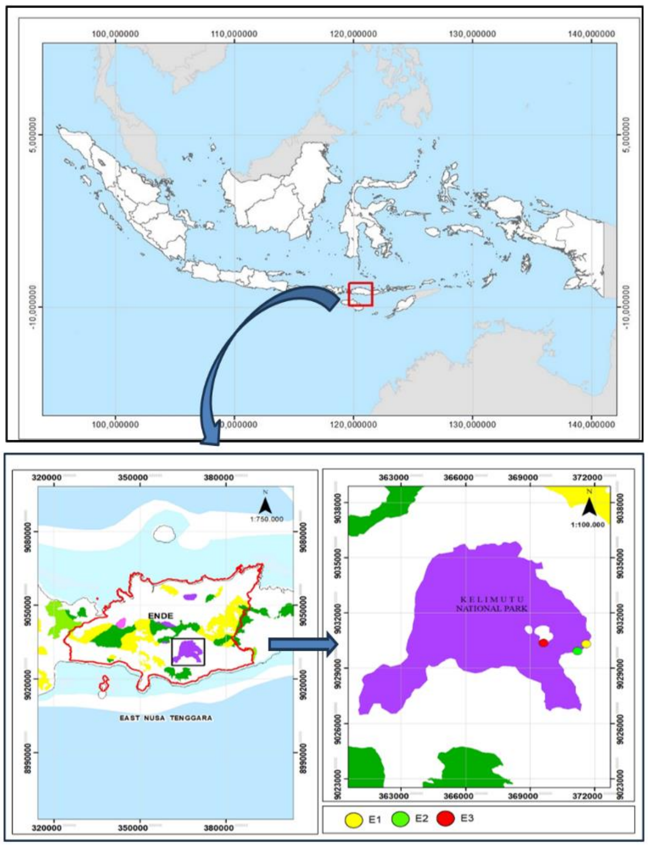

Fig. 1: Study sites at Danau Kelimutu National Park, Ende Island, Province of East Nusa Tenggara. E1 (yellow circle) represents the research locus at an altitude of 1247 masl, E2 (green circle) represents the research locus at an altitude of 1321 masl and E3 (red circle) represents the research locus at an altitude of 1585 masl

The ANOVA test indicated that the population of the functional groups (cellulolytic microbes, nitrogen fixers, phosphate solubilizers) was significantly determined by the altitudes, rhizosphere assemblages, and their interaction (Table 3a). Further analysis (Table 3b) demonstrates that the rhizosphere assemblage produced a stronger influence on the microbe colonization than the altitudes or their interactions. Altitudes and the interaction with rhizosphere assemblages only influence the colonization of cellulolytic microbes but not the rest of the functional groups.

The shoots of edelweiss contain compounds acting as a defense system against pests, namely repellents, and phytotoxins (Table 4). Each plant produces 11–13 types of compounds in its leaves that belong to the sugar, fatty acid, and terpenoid groups found as major content in these tissues. The rhizosphere of edelweiss indicated a similar pH and available phosphate concentration as the bulk soil, but organic C and total N concentrations and exchangeable K were 6.8, 2.9 and 3.8 folds higher in the rhizosphere, compared to bulk soil, and available S levels 14.7 times lower (Table 5).

Table 2: Functional microbes in the rhizosphere and the bulk soil

Altitude	Coordinates		Samples	Cellulolytic microbes (×10^2) CFU/g dried soil	Nitrogen fixers (×10^4) CFU/g dried soil	Phosphates solubilizers (×10^2) CFU/g dried soil
	Longitude (λ)	Latitude (ϕ)				
1249	08°46'13,86"	121°49'57,48"	RS1	88±1.2	30±0.5	30±0.5
1247	08°46'13,95"	121°49'58,09"	RS2	88±0.5	40±0.5	28±0.5
1245	08°46'13,83"	121°49'58,14"	RS3	58±2.4	20±0.5	58±1.2
1246	08°46'14,33"	121°49'57,24"	RS4	44±1.2	75±3.7	20±1.2
1246	08°46'14,21"	121°49'57,15"	RS5	55±1.2	140±1.2	70±2.1
1320	08°46'26,27"	121°49'45,41"	RS6	15±0.5	21±1.6	11±0.5
1320	08°46'25,78"	121°49'45,63"	RS7	20±0.8	110±1.2	33±0.5
1323	08°46'25,42"	121°49'44,91"	RS8	30±1.2	45±1.2	15±0.5
1321	08°46'26,50"	121°49'44,63"	RS9	20±1.2	31±1.6	50±1.2
1318	08^{0}46'26,78"	121°49'45,13"	RS10	50±2.5	35±1.2	193±1.2
1583	08°46'12,55"	121°48'52,38"	RS11	25±1.2	11±1.2	65±2.1
1583	08°46'12,55"	121°48'52,38"	RS12	15±1.2	15±0.5	80±2.1
1585	08°46'12,32"	121°48'52,89"	RS13	20±1.2	40±3.3	208±6.9
1589	08°46'08,59"	121°48'53,17"	RS14	15±1.6	40±1.2	55±0.5
1589	08°46'08,59"	121°48'53,17"	RS15	33±2.1	11±1.2	83±1.6
1249	08°46'13,86"	121°49'57,48"	BS1	12.00± 1.63	7.00± 1.67	8.00±0.50
1247	08°46'13,95"	121°49'58,09"	BS2	5.67±2.05	4.00±1.63	4.33±1.25
1245	08°46'13,83"	121°49'58,14"	BS3	9.33±1.25	7.33±0.94	6.33±1.70
1246	08°46'14,33"	121°49'57,24"	BS4	10.33±2.62	6.33±1.25	7.00±1.41
1246	08°46'14,21"	121°49'57,15"	BS5	7.33±1.25	8.33±2.05	5.67±2.87
1320	08°46'26,27"	121°49'45,41"	BS6	2.33±0.47	0.00±0.00	2.67±0.47
1320	08°46'25,78"	121°49'45,63"	BS7	2.33±1.25	2.00±1.63	2.00±1.63
1323	08°46'25,42"	121°49'44,91"	BS8	0.00±0.00	0.00±0.00	2.67±0.94
1321	08°46'26,50"	121°49'44,63"	BS9	0.00±0.00	0.00±0.00	2.67±0.47
1318	08°46'26,78"	121°49'45,13"	BS10	3.33±0.47	0.00±0.00	2.67±0.94
1583	08°46'12,55"	121°48'52,38"	BS11	0.00±0.00	4.00±0.50	0.00±0.00
1583	08°46'12,55"	121°48'52,38"	BS12	0.00±0.00	3.70±0.50	0.00±0.00
1585	08°46'12,32"	121°48'52,89"	BS13	1.67±1.25	3.00±0.82	0.67±0.04
1589	08°46'08,59"	121°48'53,17"	BS14	0.00±0.00	3.33±1.89	0.00±0.00
1589	08°46'08,59"	121°48'53,17"	BS15	0.00±0.00	3.33±1.89	0.00±0.00

RS: rhizosphere soils; BS: bulk soils

Table 3: Single factor analysis of altitude, soil origin, and the interaction on the functional groups' populations

Treatments	Factors	Cellulolytic Microbes (×10^2) CFU/g dried soil	Nitrogen Fixers (×10^4) CFU/g dried soil	Phosphates Solubilizers (×10^2) CFU/g dried soil
Altitudes (A)	T1	37.90 a	33.96 a	23.87 a
	T2	12.83 b	25.93 a	30.43 a
	T3	12.23 b	12.03 a	50.37 a
Soil origin (S)	RS	38.02 a	44.49 a	66.82 a
	BS	3.95 b	3.47 b	2.96 b
A×S	T1RS	66.86 a	61.33 a	41.53 ab
	T2RS	25.33 b	48.47 ab	60.73 ab
	T3RS	21.86 bc	23.66 ab	98.20 a
	T1BS	8.93 bc	6.60 b	6.20 b
	T2BS	2.60 c	0.40 b	2.54 b
	T3BS	0.33 c	3.40 b	0.13 b

T: elevation; RS: rhizosphere soils; BS: bulk soils. $P < 0.000$ (α=0.05)

Discussion

In this study, an edelweiss secreted approximately 370 compounds classified into five functions, collectively called root exudates. The rhizosphere consists of 24% antimicrobial (organic compounds, volatile organic compounds, and various fatty acids), 18% energy sources (alcohols and amino acids), 44% nutrient acquisition and community shape (organic acids), 8% stress responses, induction of system resistance (volatile organic compounds and terpenoids), and 6% allelochemicals (steroid alkaloids, terpenes, terpenoids, and aldehydes) (Fig. 2).

The secretion of energy sources (sugars, alcohol sugars, amino acids, etc.) found in the edelweiss rhizosphere (18%) (Fig. 2), is much lower than annual plants (40%), or perennial plants (70%) (Dey and Sengupta 2020) of their photosynthetic products into the root zone. In general, plant rhizodeposition is about 800–4500 kg C/ha/year (Kuzyakov and Domanski 2000). Amino acids play important roles as nutrient sources, binders of inadequately soluble mineral elements, and chemo-attractants to microbes (Haichar *et al*. 2014). However, in the non-rhizosphere soil without the impact of root activity, only 71 compounds were found; almost all (90%) were antimicrobial (Fig. 2), while sugars functioning as C and energy sources for microbes were only found circa 2%. This is why functional groups rarely colonized the bulk soil of this study site. The rare of soil microbes found in the site also may resulted of the lower

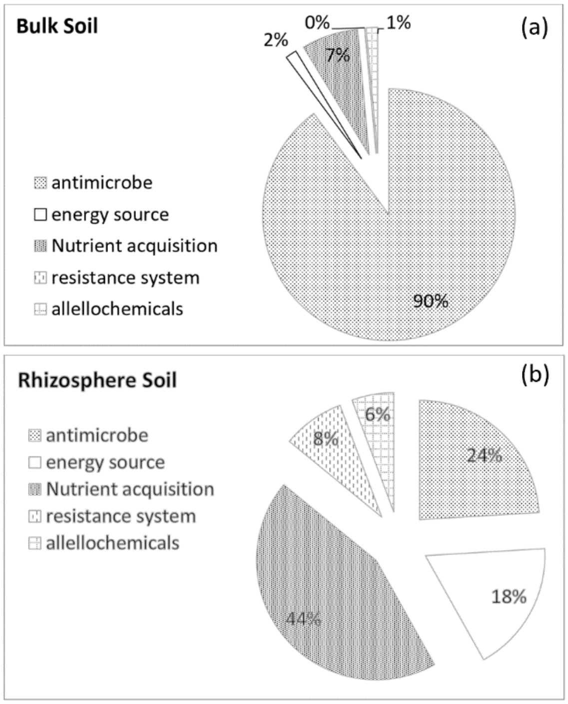

Fig. 2: Composition of compounds analyzed of the Edelweiss rhizosphere (a) and the bulk soil (b). The compounds can be classified into fives based on their functions: antimicrobial, energy source, nutrient acquisition, stress response, and allelochemicals. Most of the chemicals are found to be antimicrobial, followed by nutrient acquisition. The bulk soil cannot be found in chemical functioning as a stress response and resistance system

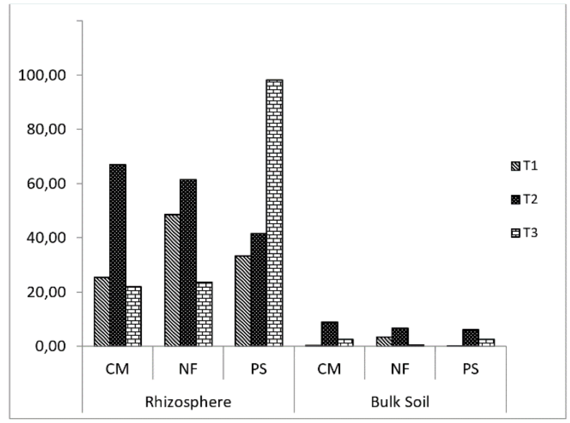

Fig. 3: Averaged population of functional groups isolated from the soil root zone of edelweiss and the bulk soil. CM: cellulolytic microbes; NF: nitrogen fixers; PS: phosphate solubilizers. T1: elevation 1200s masl; T2: elevation 1300s masl; T3: elevation 1500s masl

temperatures in the high altitude (Lazzaro *et al.* 2015), young volcanic soil with oligotrophic, harsh, nutrient-limited conditions, coarse texture, poor water holding capacity, and tiny solum (Ciccazzo *et al*. 2014; Rime *et al.* 2015) that inhibit biological activities (Donhauser and Frey 2018).

Edelweiss secreted 44% of compounds functioning as nutrient acquisition and community shaping (Mimmo *et al*. 2014; Zhalnina *et al*. 2018) that may respond to the biotic and abiotic stress in the environment (Weston *et al*. 2012; Haichar *et al*. 2014). It also supports edelweiss to grow in the limited N but excessive S in the soil (Table 5), which is presumably due to volcanic activity. Interestingly, nitrogen fixers isolated from the edelweiss rhizosphere was identified as *Azospirillum thiophylum*, which is also recognized as capable of utilizing excess S content in soil as an energy source (Lavrinenko *et al.* 2010). The S content in the rhizosphere decreased by more than 14-fold compared to the bulk soil (Table 5).

Compounds classified as organic acids were the most abundant in the rhizosphere of Edelweiss (Fig. 2a,b). Low molecular weight organic acids are of considerable interest among known root exudates due to their essential roles in stimulating and controlling microbial growth (Sasse *et al*. 2018; Cotton *et al.* 2019), detoxifying potentially toxic metals (e.g., Al^{3+}), mobilizing poorly soluble nutrients e.g., P, Fe and Zn (Shen *et al*. 2011; Vocciante *et al*. 2022), and speeding up mineral weathering (Oburger *et al*. 2009; Haichar *et al*. 2014).

In the edelweiss rhizosphere functional groups such as N fixers (NF), cellullolitic degraders (CP), and P solubilizers (PS) can be isolated (Fig. 3; Table 2). Edelweiss allocates 18% of rhizodeposition as alcohols and amino acids (Fig. 2; Table 1) that may act as carbon and nitrogen suppliers for soil microbes and signals to attract or repel microbes (Wu *et al.* 2017b; Praeg *et al.* 2019).

We hypothesize that Edelweiss recruits these microorganisms to provide the nutrients required for survival and growth. The soil communities isolated from the Edelweiss rhizosphere differed markedly from those in the non-rhizosphere soil (Fig. 3), proving the hypothesis. This study discovered that functional microbial populations (CM, NF, and PS) were tenfold more abundant in the Edelweiss rhizosphere than in the bulk soil area (Table 2). Because soil microorganisms contribute significantly to biogeochemical, such as the C, N, S and P cycles, rhizospheres in edelweiss plants are expected to improve soil formation processes in mountainous regions. Increasing the process of soil formation enhances edelweiss growth, thereby facilitating their adaptation to mountain ecosystems. Especially, edelweiss has to deal with the excessive S, and they employ *A. thiophyllum* to assimilate N and S.

Results showed that the selected functional of groups (CM, NF and PS) were primarily determined by the ecosystem from which the sample was obtained (Table 3). The presence of these functional microbial groups is affected by the interaction between altitude and the ecosystem from which the sample is derived (Table 3). It is confirmed by Donhauser and Frey (2018) that, in terms of microbial succession in alpine ecosystems, plant establishment stabilizes microbial community structures,

Table 4: Averaged content of functional chemical compounds identified in leaves

Altitude	No. of identified compounds	Group	Dominant compounds	Role	Relative abundance (%)
T1	13	Fatty acid	Hexadecanoic acid; 6-Octadecanoic acid methyl ester(Z)-(CAS) methylpetroselinate; 1,2-Benzedecarboxilic acid dioctyl ester (CAS)dioctyl ptalate	Repellent	4.1–6.76
		Terpenoid	Phytol	Phytotoxin	5.51
		Sugar	Isosorbid	Phytotoxin	4.85
T2	12	Fatty acid	Hexadecanoic acid	Repellent	4.95
		Amide	Methanamide	Repellent	4.71
		Hydrocarbon	Tridecane (CAS) n-Tridecane	Repellent	4.17
		Phenolic	Undecane, 2-methyl- (CAS) 2-Methylundecane	Phytotoxin	4.81
		Terpenoid	Phytol	Phytotoxin	4.23
		Phenilpropanoid	Hexyl cinnamic aldehyde	Phytotoxin	4.13
T3	11	Fatty acid	Hexadecanoic acid; Decanoic acid; 1,2- Benzenedicarboxylic acid, dioctyl ester (CAS) Dioctyl phthalate	Repellent	4.11–8.77
		Terpenoid	Phytol	Phytotoxin	4.54
		Sugar	Isosorbid	Phytotoxcin	7.01

Table 5: Site descriptions and chemical soil characteristics, including annual mean temperature (Temp), pH, total organic carbon, total N, C/N ratio, and S, P and K availability

Altitude	Temp.(ºC)	Rhizosphere						Bulk Soil					
		pH H_2O (1:5)	OC (%)	Total N (%)	P avb (ppm) Bray1	K avb (ppm) Bray1	S avb (ppm)	pH H_2O (1:5)	C org (%)	Total N (%)	P avb (ppm) Bray1	K avb (ppm)	S avb (ppm)
1200s	14.2	6.77	4.75	0.51	39.5	16.5	35.7	6.43	0.82	0.09	37.2	3.2	664.2
1200s	13.8	6.45	4.85	0.52	40.6	17.9	29.1	6.12	0.94	0.11	35.1	11.5	478.0
1350s	12.6	6.66	3.16	0.38	37.7	19.4	74.4	6.31	0.43	0.18	35.5	7.3	821.0
1350s	10.8	6.62	3.98	0.33	42.2	21.2	44.7	6.42	0.77	0.07	36.2	5.2	772.3
1500s	8.9	6.38	4.41	0.62	36.8	18.4	31.4	6.36	0.52	0.14	41.2	3.7	561.4
1500s	9.3	6.58	5.12	0.49	44.3	22.3	57.3	6.64	0.39	0.16	39.4	8.4	706.3

Avb: available; N total (Kjeidhal); Corg (Walkey & Black); P& S NH_4POAc pH 4. Means are from n = 5

which are further built by interactions with these plants' rhizosphere. Thus, the structure of plant microbe community is the result of a series of forward and reverse interactions between plants, microorganisms, and their physical, chemical, and environmental conditions (Pang *et al.* 2021).

In this study, the plant tissue of edelweiss contained terpenes, phenolics, and several groups of compounds (Table 4) that can be classified as plant secondary metabolite (PSMs) (Huang *et al.* 2018; Kessler and Kalske 2018), which generally act as pest repellents and phytotoxins important in plant defense systems. The study also found some forms of flavonoids and phytol as antimicrobial against various pathogens (Bharathy *et al*. 2012; Mujeeb *et al*. 2014; Górniak *et al*. 2019), terpenoids important phytohormons (Chen *et al*. 2019; Wang and Niu 2019) and involved in plant symbiosis (Xie *et al*. 2010).

Some PSMs, such as flavonoids, are not only linked to the response of plant-microbe symbioses (e.g., arbuscular mycorrhiza, ectomycorrhiza, rhizobia, and actinorhiza symbioses) but also with quorum-sensing (QS) persuaders for communication by many microbes (Pang *et al.* 2021). Flavonoids are also associated with *In vitro* antimicrobial activity against various pathogenic microorganisms (Górniak *et al.* 2019). Terpenoids, crucial root-specific metabolites (Wang and Niu 2019), have been extensively studied as phytohormones and compounds involved in plant symbiosis (Xie *et al.* 2010). This study is in line with Zhalnina *et al.* (2018) that the blend of root exudates secreted (Table 1) with the functional groups inhabiting the rhizosphere (Table 2) leads Edelweiss to succeed in colonizing and surviving the cool and harsh ecosystem of Kelimutu Mountain and creates mono savannah formation.

Conclusion

To colonize and survive in the shallow and harsh soil of the Kelimutu Volcanic Mountain, *E. longifolia* produces a suitable rhizosphere. The most abundant root exudate found in the edelweiss soil was organic acid, which is recognized to contribute to soil community shaping and nutrient acquisition. The functional group colonization is strongly determined by the rhizosphere assemblage than the altitude properties. By rhizosphere formation C, K and N availability dramatically improved whilst the availability of S was significantly reduced. To deal with the excessive S content, *A. thiophylum* in the rhizosphere of edelweiss was found to assist in assimilating S and providing N. Rhizosphere assemblages also produce compounds identified as allelochemicals that are assumed to suppress the growth of other plants; consequently, they always form a monoculture savannah. In the rhizosphere, phytotoxins may play an important role against pests and diseases in the soil. Further, the rhizosphere also supports depositing some compounds classified as insect repellents in their leaf tissues.

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Author Contributions

EW, AS, RSBI, and GP planned the experiments and conducted field work; MW, NEL, SU, TK, NY, and S conducted lab works and interpreted the results; EW, AS, RSBI, GP, MW, NEL, SU, TK, NY, and S made original manuscript and revised one; MVN reviewed final manuscript; and WD, M, SAF statistically analyzed the data and made illustrations.

Conflicts of Interest

All authors declare no conflict of interest

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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