

**ENVIRONMENTAL EFFECTS OF VOLCANIC
ERUPTIONS: A MULTIDISCIPLINARY STUDY OF
TEPHRA IMPACTS ON PLANT AND SOIL**

INGA TARASENKO

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Promoteurs:

PROF. PIERRE DELMELLE, UCL/ELI/ELIE, Belgique
PROF. SOPHIE OPFERGELT, UCL/ELI/ELIE, Belgique

Membres du jury:

PROF. BRUNO DELVAUX (Président), UCL/ELI/ELIE, Belgique
PROF. STANLEY LUTTS (Secrétaire), UCL/ELI/ELIA, Belgique
PROF. CHARLES BIELDERS, UCL/ELI/ELIE, Belgique
PROF. STEEVE BONNEVILLE, ULB, Belgique

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SUPERVISORS:

Prof. Pierre Delmelle
Prof. Sophie Opfergelt

JURY MEMBERS:

Prof. Bruno Delvaux (President)
Prof. Stanley Lutts (Secretary)
Prof. Charles Bielders
Prof. Steeve Bonneville

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If we knew what it was we were doing,
it would not be called research, would it?

— A. Einstein

ABSTRACT

Volcanic active regions are often intensively cultivated and densely populated as they are typically endowed with fertile soils developed from volcanic eruption products. However, these regions often receive tephra deposition, including ash (< 2 mm) and scoria (> 2 mm), which threaten the natural and cultivated ecosystems. This PhD study aims at unravelling some of the complex interactions that drive the impacts of tephra on soil physical properties, soil microorganisms and crop plants. Surface crusting of fresh ash deposits impedes water infiltration in soils and increases runoff. Using an original laboratory experiment, I highlighted that structural crust formation is the result of particle reorganisation at the surface of the ash deposit subjected to raindrop impact. The grain size distribution of the ash material exerts a strong control on crust development. In a second study, I investigated the early stage colonisation of tephra by fungi. Using various techniques, I showed that freshly-deposited tephra represent an attractive ecological niche for ectomycorrhizal fungi from the underlying soil. Such colonisation may accelerate revegetation and soil formation of a tephra-affected area. Finally, the effects of an ash deposit on the growth and physiology of tomato and rice plants were examined for the first time. Experiments conducted in a phytotron revealed that ash deposition on foliage alters photosynthesis and decreases plant biomass. Multiple applications of ash produce a stronger and longer effect on plants than a single event. The intensity of the ash effects is likely plant-specific. Overall, this PhD study provides novel insights into the role of ash as an agent of environmental disturbances. It paves the way for further investigations aimed at informing the development of adequate agricultural practices that can decrease the vulnerability of rural communities exposed to volcanic tephra.

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CONTENTS

I INTRODUCTION

1	GENERAL INTRODUCTION	3
1.1	Context	3
2	STATE OF THE ART	15
2.1	Overview of a volcanic eruption	15
2.2	Physical and chemical properties of tephra	16
2.3	Environmental effects of tephra	21
2.3.1	Residence time of tephra on soil	23
2.3.2	General physical effects	25
2.3.3	Chemical effects	28
2.3.4	Biological effects	29
2.4	Tephra deposition on vegetation	32
2.4.1	Residence time on vegetation	32
2.4.2	Physical effects on vegetation	32
2.4.3	Chemical effects on vegetation	33
2.4.4	Physiological effects on vegetation	36
2.5	Thesis outline	40

II DATA CHAPTERS

3	SURFACE CRUSTING OF A VOLCANIC ASH DEPOSIT UNDER SIMULATED RAINFALL	55
3.1	Abstract	55
3.2	Introduction	56
3.3	Materials and methods	57
3.3.1	Experimental configuration	57
3.3.2	Post-experiment analyses	60
3.4	Results	63
3.4.1	Micro-topography	64
3.4.2	Micro-morphology	65

3.4.3	Porosity measurements	75
3.5	Discussion	76
3.5.1	Changes in the surface micro-topography of the ash deposit	76
3.5.2	Evidence for surface crusting of the ash deposit	77
3.5.3	Mechanisms of crust formation at the surface of the ash deposit	78
3.6	Concluding remarks	83
4	FRESH TEPHRA DEPOSITS PROVIDE A HABITAT FOR RAPID FUNGI COLONIZATION: A CASE STUDY FROM MT. ETNA	93
4.1	Introduction	93
4.2	Materials and methods	97
4.2.1	Study site	97
4.2.2	Sampling strategy	101
4.2.3	Tephra and soil characterization	102
4.2.4	Imaging of tephra	103
4.2.5	Quantification of fungal biomass: ergosterol extraction and HPLC analysis	105
4.2.6	Molecular analyses	107
4.2.7	Statistical analysis	112
4.3	Results	113
4.3.1	Tephra and soil general properties	113
4.3.2	Fungal colonization of freshly-deposited tephra	115
4.4	Discussion	127
4.4.1	Evidence for fast fungal colonization of freshly-deposited tephra	127
4.4.2	Variability of fungal distribution over the volcanic soil profile	131
4.4.3	Identification and origin of fungi in the freshly-deposited tephra	134
4.4.4	Tephra as potential ecological niche for fungi	137
4.5	Conclusions	139

5	EFFECTS OF A VOLCANIC ASH DEPOSIT ON THE GROWTH AND LEAF PHYSIOLOGY OF TOMATO PLANTS (<i>solanum lycopersicum</i>)	155
5.1	Abstract	155
5.2	Introduction	156
5.3	Materials and methods	158
5.3.1	Plant material and growing conditions	158
5.3.2	Ash deposition scenarios	159
5.3.3	Leaf temperature, photosynthesis- and plant growth-related parameters	160
5.3.4	Biochemical parameters	164
5.3.5	Statistical analysis	167
5.4	Results	167
5.4.1	Plant growth	169
5.4.2	Photosynthesis-related parameters	169
5.4.3	Soluble sugars and protein contents	173
5.4.4	Oxidative stress-related parameters	176
5.5	Discussion	179
5.5.1	Effects of ash deposition on plant growth	179
5.5.2	Effects of ash deposition on plant physiology and biochemical parameters	180
5.5.3	Single versus multiple ash deposition	186
5.6	Conclusions	187
6	EFFECTS OF A VOLCANIC ASH DEPOSIT ON THE GROWTH AND LEAF PHYSIOLOGY OF RICE PLANTS (<i>pokkali</i>)	197
6.1	Abstract	197
6.2	Introduction	198
6.3	Materials and Methods	200
6.3.1	Plant material and growing conditions	200
6.3.2	Ash treatment	201
6.3.3	Leaf temperature, photosynthesis-, plant growth-related and biochemical parameters	201
6.4	Results	202
6.4.1	Plant growth and development	204

6.4.2	Photosynthesis-related parameters	204
6.4.3	Soluble sugars and protein content	209
6.4.4	Oxidative stress-related parameters	212
6.5	Discussion	214
6.5.1	Effects of ash deposition on plant growth	214
6.5.2	Effects of ash deposition on plant physiology and biochemical parameters	216
6.5.3	Single versus multiple ash deposition	221
6.6	Conclusions	222
 III SUMMARY AND PERSPECTIVES		
7	CONCLUSIONS AND PERSPECTIVES	233
7.1	Mechanisms of surface crusting of an ash deposit	233
7.2	Colonization of a coarse-grained tephra deposit by fungi at Mt. Etna	234
7.3	Impact of ash deposition on crop plants	236
 IV SUPPLEMENTARY MATERIAL		
A	SUPPLEMENTARY FOR CHAPTER 1	245
A.1	Objectives of the EU-FP7 ITN project Vertigo	245
B	SUPPLEMENTARY FOR CHAPTER 3	249
B.1	Sample description	249
B.2	Particle size distribution	249
B.3	Ash leachate analysis	252
B.4	Micro-topography and micro-morphology measurements	253
B.5	Porosity measurements	255
B.6	Python script for porosity measurements	259
C	SUPPLEMENTARY FOR CHAPTER 4	263
D	SUPPLEMENTARY FOR CHAPTER 5	267
E	SUPPLEMENTARY FOR CHAPTER 6	271
F	CONFERENCES AND MISSIONS ABROAD	275
F.1	Conferences	275
F.2	Missions abroad and contributions to other projects	276

LIST OF FIGURES

Figure 2.1	Schematic representation of ascent of the gas-particle mixture in the volcanic plume to the height of neutral buoyancy and dispersion of the volcanic cloud ([70]).	17
Figure 2.2	Cartoon diagram illustrating the receiving environments of tephra that undergone transition through the volcanic plume and cloud (E) before environmental interaction occurs. Tephra is then emitted into the atmosphere (A). Deposition will then carry the particles onto vegetation surfaces (V), such as forests (Vf), cropland (Vc) and grasslands (Vg). Tephra may also be deposited onto land surfaces (T), such as soil (Ts), wetlands (Tw) or onto snow or ice (S). Particles falling into the aquatic system (W) may interact with the ocean and ocean sediments (Wo), lakes and lake sediments (Wl) and river and river beds (Wr) ([8]).	18
Figure 2.3	The chemical classification of the volcanic rocks based on total alkalis versus silica (TAS). Superimposed are the boundaries that delineate different rock types according to the International Union of Geological Sciences (IUGS) ([51]) with the names of volcanic and selected plutonic rocks types. Taken from [51].	19

Figure 2.4	Scanning electron microscopy images of the tephra particles with different shapes: spherical tephra particle (a) ([38]); elongated tephra particle (b), tephra particle with sharded bubble walls (c), blocky tephra particle (d) ([47]).	21
Figure 2.5	Simplified cartoon showing the tephra fall depositions and compartments that may be affected by tephra, including animals, vegetation, soil and microorganisms. Adapted from [17].	23
Figure 2.6	Three forms of wind erosion: creep (rolling) of particles with diameter above 0.5 mm; saltation (bouncing) of particles with diameter range between 0.01-0.5 mm and suspension of eroded particles by particles subjected to saltation. Image is taken from www.milford.nserl.purdue.edu	24
Figure 2.7	Diagram representing the forms of water erosion: sheet erosion (1); rill erosion (2); gully and ravine erosions (3). Diagram is taken from www.next-book.co	25
Figure 2.8	Potential impacts of tephra on soils.	27
Figure 2.9	Photographs of tephra from the eruption of Tungurahua volcano showing the colour differences with respect to the fine content (<63 µm) of the tephra ([36]).	27
Figure 2.10	Schematic representation of the $\text{CO}_2/\text{H}_2\text{CO}_3/\text{HCO}_3^-$ - weathering/transport cycle, occurring between the tephra and Oa (organic-rich) horizon of the buried soil. C_B is a base cation. ([24]).	31

Figure 2.11	(a) - The same forest site in 1980 after the Mount St. Helens eruption, USA, and in 2013. The complete burial of understory by tephra (i. e. ash particles) and survived trees after the ash deposition Joe Antos and Dylan Fischer, www.cbsnews.com ; (b) - Broken trees due to tephra (i. e. ash particles) deposition in the Tungurahua province after the Tungurahua eruption in August, 2016 El Comercio www.elcomercio.com	35
Figure 2.12	(a) - The tree leaves in the western sector of Tungurahua erupted on 5 November, 2015. V. Lema (IG-EPN) www.igepn.edu.ec ; (b) - Crops affected by the tephra (i. e. ash particles) fall from the eruption of Tungurahua on November 14th, 2015 X. Parra (IG-EPN) www.igepn.edu.ec	35
Figure 2.13	(a) - Vegetation reappearance through the cracks of cemented tephra (i. e. ash particles) from the eruption of Mt. St. Helens in 1980 Barry Kough of the Lewiston Tribune www.inland360.com ; (b) - Tomato plants presumably damaged due to acid release from tephra (i. e. ash particles) after the eruption of Mt. Merapi, Indonesia in June, 2006 ([86]).	36
Figure 3.1	Schematics of the 5-cm diameter polyvinyl chloride (PVC) column used to hold the ash deposit (A); the set up used for reconstructing the ash deposit (B); the rainfall simulator placed 4 m above the ash-containing column (C).	58
Figure 3.2	Schematic representation of the micro-topography of the ash deposit surface subjected to raindrops: micro-peak (<i>Micro-peak counts</i> (P_c)), micro-crater (<i>Micro-crater counts</i> (C_c)) and vertical distance between top of a micro-peak and bottom of a micro-crater (<i>Maximum crater to peak height</i> (R_{cp})).	63

Figure 3.3	Photographs of the surface of Mt. Merapi, Indonesia (MER) (A), San Cristobal, Nicaragua (SC) (B) and Eyjafjallajökull volcano, Iceland (EYJA) (C) ash deposits after exposure to 5, 10, 15 and 20 mm of rainfall. The white arrows point to ash pellets formed at the MER and SC deposits surface immediately after rainfall initiation. . . .	65
Figure 3.4	Optical microscope images (stitched) of the thin cross sections of the MER ash deposit after exposure to 5 mm (A), 10 mm (B), 15 mm (C) and 20 mm (D) of rainfall. The rectangle outlined in white highlights the location of the thin layer comprised of tightly-packed ash particles. The representativity of the observed particle reorganisation along the sample is demonstrated through the image of ~ 4 cm cross section of the MER deposit exposed to 20 mm of rainfall (E). . .	67
Figure 3.5	Scanning Electron Microscopy (SEM) images of polished cross sections of the MER ash deposit after exposure to 5 (A), 10 (B) and 20 mm (C) of rainfall. The red dashed lines delineate the near-surface layer affected by particle reorganisation in micro-craters. The ash material located below this layer corresponds to the unaffected bulk deposit.	68

- Figure 3.6 Optical microscope images (stitched) of the thin cross sections of the SC ash deposit after exposure to 5 mm (A), 10 mm (B), 15 mm (C) and 20 mm (D) of rainfall. The rectangle outlined in white highlights the location of the thin layer comprised of tightly-packed ash particles. The representativity of the observed particle reorganisation along the sample is demonstrated through the image of ~ 4 cm cross section of the SC deposit exposed to 20 mm of rainfall (E). . . . 70
- Figure 3.7 SEM images of polished cross sections of the SC ash deposit after exposure to 5 (A), 10 (B) and 15 mm (C and D) of rainfall. The red dashed lines delineate the near-surface layer affected by particle reorganisation in micro-craters (A), (B) and (D), and in micro-craters and on micro-peaks (C). The ash material located below this layer corresponds to the unaffected bulk deposit (E). . 71
- Figure 3.8 Optical microscope images (stitched) of the thin cross sections of the EYJA ash deposit after exposure to 5 mm (A), 10 mm (B), 15 mm (C) and 20 mm (D) of rainfall. The rectangle outlined in white highlights the location of the thin layer comprised of tightly-packed ash particles. The representativity of the observed particle reorganisation along the sample is demonstrated through the image of ~ 4 cm cross section of the EYJA deposit exposed to 20 mm of rainfall (E). . 73

Figure 3.9	SEM images of polished cross sections of the EYJA ash deposit after exposure to 5 (A), 10 (B) and 15 mm (C) of rainfall. The red dashed lines delineate the near-surface layer affected by particle reorganisation in micro-craters (A), (B) and (C). The ash material located below this layer corresponds to the unaffected bulk deposit (D). . . .	74
Figure 4.1	Map of the study sites in 2014 and 2015. Red circle - sampling site (GPS coordinates for sampling site in 2014 : UTM x=0504020, y=4181577; GPS coordinates for sampling site in 2015: UTM x = 0504050, y = 4181572).	98
Figure 4.2	Schematic representation of sampling site and procedure (1). Size of the plot (10 × 10 m) and distribution of soil profiles (2 m) sampled. The samples of the various tephra (>2 mm, TT, BT, and <2 mm, T) and 'buried' soils (S1, S2 and S3) were collected up to 40 cm depth. The sampling was performed annually in September 2014 and 2015. Twenty soil profiles were dug within a 10 × 10 m plot. Composite samples were made of material collected from five soil profiles located at distance of 2 m from each other. In total, four composite field replicates were sampled from six tephra and soil layers, resulting in 24 samples. Sampling site photo: Sicily, Etna (2). Coniferous forest. Elevation: 1755 m above-sea-level.	99

Figure 4.3	Schematic representation and photo of the volcanic soil profile, where TT (top 10 cm) + BT (bottom 10 cm) is the tephra deposited on November 23, 2013. *The colours used here to illustrate the different layers in volcanic soil are kept in Section 4.3. The soil horizons have the same characteristics in all sites.	100
Figure 4.4	O'GeneRuler 1 kb Plus Deoxyribonucleic acid (DNA) Ladders are on the left and right-end side of each gel image (a-d). In between the ladders, each lane represents individual replicates within each horizon -labels on top- for 2014 and 2015. Images of gel were analysed by GelAnalyzer software. In Figure 4.4e, the profile windows show the intensity of the DNA ladder and TT and BT samples from 2014 and 2015 (blue line is the background level). The vertical axis in Fig. 4.4e is the distance from the lane's start position in pixels, while the horizontal axis shows the intensity values. The ladder on the left of Fig. 4.4e is the reference lane image from supplier of O'GeneRuler 1 kb Plus DNA Ladder (in base pair - bp).	109
Figure 4.5	Characterization of the tephra and soil (<2 mm fraction) samples: total reserve in bases (Total reserve in bases (TRB), cmolc kg^{-1}) (a). Dotted vertical line represents the TRB value of the fresh tephra deposited on November 23, 2013; soil acidity ($\text{pH-H}_2\text{O}$) (b); total organic carbon (Total organic carbon (TOC), g kg^{-1}) (c); total nitrogen (Total nitrogen (TN), g kg^{-1}) (d); total organic matter (Total organic matter (TOM), %) (e), The ratio of total organic carbon to total nitrogen (C/N) (f).	114

Figure 4.6	An insert: representative colonized tephra particle from the BT layer (~ 2.5 cm in diameter) showing large encrustation of fungal mycelium clearly visible in fluorescence microscopy by staining with WGA-FITC, a dye highly specific of chitin (a). In contrast, TT tephra particle surface (insert B) is devoid of fungal encrustation. A few hypha are present within vesicular tephra structure as evidenced by WGA-FITC staining (b). Note that both tephra were sampled in 2014. (c and d) Electron micrographs of the surface of BT tephra collected in 2014 showing extensive fungal mycelium (d) and a fungal structure penetrating the mineral substrate (c). Electron micrographs of the thin sections of BT (e) and TT (f) tephra collected in 2014 showing fungal colonization (e) and no evidence of fungal colonization (f).	117
Figure 4.7	Fungal biomass (Fungal biomass (FB)) profile with soil depth in 2014 and 2015. FB is the mean of four sample replicates. Error bars represent SD from the mean.	118
Figure 4.8	O'GeneRuler 1 kb Plus DNA Ladder representing the known quantities of nucleic acids (0.1 µg per µL DNA ladder) (a). This calibration DNA Ladder bands varies from 75 to 20000 bp. Note that the same ladder is present on the right and left-end side of each gel. Gels of DNA extracted from tephra and buried soils samples collected in 2014 and 2015 respectively (b and c). Lanes from left to right correspond to following replicates: TT, BT, S1, T, S2 and S3.	119

- Figure 4.9 Total amount of extracted DNA (from both bacterial and fungal communities) quantified by the 1D gel electrophoresis image analysis software GelAnalyzer and analysis of densitometry profiles of GelRed-stained DNA gels. Total amount of DNA is the mean of two sample replicates for 2014 and four sample replicates for 2015. Error bars represent SD from the mean. 120
- Figure 4.10 O'GeneRuler 1 kb Plus DNA Ladder representing the known quantities of nucleic acids (0.1 µg per µL DNA ladder) (a). This calibration DNA Ladder bands varies from 75 to 20000 bp. Note that the same ladder is present on the right and left-end side of each gel. According to this ladder, the estimated size of the amplicons is 700 bp. Polymerase chain reaction (PCR)-Denaturing gradient gel electrophoresis analysis (DGGE) products of the samples collected in 2014 and 2015, respectively, amplified with ITS1 Myers/ITS4-KY01 primers for the ITS1-5.8SrRNA-ITS2 region present in all members of fungal community (b and c). Polymerization temperature is 47 °C. Lanes correspond to the following field replicates: TT, BT, S1, T, S2 and S3; H₂O – blank. 124
- Figure 4.11 DGGE profiles of fungal amplicons of the same size, but different sequences, representing communities in tephra and soils in 2015 along the volcanic soil profile (TT, BT, S1, T, S2 and S3) (a). White rectangles on the gel lanes indicate position of individual bands, i. e. ribotypes. Numbers of DGGE ribotypes in tephra and soil collected in 2015 (b). Mean ± SD of four field replicates are shown. 125

Figure 4.12	DGGE lanes of tephra and soil replicates from 2015 (a). Heatmap based on Jaccard estimations of fungal ribotype similarity in tephra and soil collected in 2015 as measured from DGGE bands (1.0 corresponds to the ribotype complete similarity)(b).	126
Figure 5.1	Map of sampling site. The sampling site is shown with red dot.	160
Figure 5.2	Photographs of the 135 cm-high polyvinyl chloride (PVC) tube used for ash application to the plants (left); ash (indicated by black arrow) applied to the plants through a 250 µm mesh resting upon the top of the tube (right).	161
Figure 5.3	Schematics of the scenarios of ash application corresponding to a single event (a, S1) or to a multiple event (b, S2). In S1, plants received 90 g of ash on, day 0, while in scenario 2, plants received 30 g of ash at days 0, 3 and 8. Leaf temperature, physiological and plant-growth-related measurements were performed 8 times during a two-week treatment. The position of the leaf used for leaf temperature, physiological and plant-growth-related measurements is indicated with a red cross and corresponds to a leaf n° 4 (numbered acropetally) for day 1 until day 4, and to a leaf n° 5 for day 7 until day 14. Hence, leaf n° 5 appeared after the ash treatment in scenario 1, while it received two ash applications in S2. The images of tomatoes were taken from www.nl.depositphotos.com/111421292	162

- Figure 5.4 The height of the tomato shoots, measured from the interface of roots and shoots up to the highest leaf, and the length of the tomato roots. Results are the means of six sample replicates (a); biomass of dry tomato shoots and roots. Biomass is the mean of three sample replicates (b). Error bars represent SE from the mean. 168
- Figure 5.5 Water content in tomato shoots and roots. Water content is the mean of three sample replicates. Error bars represent SE from the mean. 170
- Figure 5.6 Net photosynthesis (a); instantaneous transpiration (b); stomatal conductance (c) and water use efficiency (d) of the controls, S1 and S2 leaves. Results are the means of six sample replicates. Error bars represent SE from the means. 173
- Figure 5.7 Temperature of the fully expanded leaves, obtained by IR-camera. Leaf temperature is the mean of six sample replicates. Error bars represent SE from the means. 174
- Figure 5.8 Total soluble sugar content (a) and total soluble protein content (b) in the controls, S1 and S2 tomato shoots and roots on days 1 – 4 and days 7, 9, 11 and 14. Results are the means of three sample replicates. Error bars represent SE from the means. Means with different letters are significantly different (Tukey's test, $p < 0.05$). . . 175
- Figure 5.9 MDA in the controls, S1 and S2 tomato shoots and roots on days 1 – 4 and days 7, 9, 11 and 14 (a); carbonyl contents in the controls, S1 and S2 tomato shoots on days 1 – 4 and days 7, 9, 11 and 14 (b). Results are the means of three sample replicates. Error bars represent SE from the means. Means with different letters are significantly different (Tukey's test, $p < 0.05$). 177

Figure 5.10	Hydrophilic (<i>HAA</i>) and lipophilic (<i>LAA</i>) antioxidant activity in tomato shoots on days 1 – 4 and days 7, 9, 11 and 14. Results are the means of three sample replicates. Error bars represent SE from the means. Means with different letters are significantly different (Tukey’s test, $p < 0.05$).	178
Figure 5.11	Principal Component Analysis of variability of parameters, tested for assessing growth and physiological condition of the controls and ash-treated plants (S1 and S2) (a); Vectors of the considered variables showing the correlation between them (b).	181
Figure 6.1	Scenarios of ash application corresponding to one single event (a, S1) or to a multiple-event (b, S2). In scenario 1, plants received 180 g of ash on day 0, while in scenario 2, plants received 60 g of ash on days 0, 3 and 8. Leaf temperature, photosynthesis- and plant-growth-related measurements were performed 8 times during a two-week treatment. The position of these measurements is indicated with a red cross and corresponds to a leaf n° 5 (numbered acropetally) for day 1 until day 9, and to a leaf n° 6 for days 11 and 14. Hence, leaf n° 6 appeared after the ash treatment in scenario 1, while it received two ash applications in scenario 2.	203
Figure 6.2	The height of the rice shoots, measured from the interface of roots and shoots up to the highest leaf, and the length of the rice roots. Results are the means of six sample replicates. Error bars represent SE from the means.	205
Figure 6.3	Biomass of dry rice shoots and roots. Biomass is the mean of three sample replicates. Error bars represent SE from the means.	206

Figure 6.4	Water content in rice shoots and roots. Water content is the mean of three sample replicates. Error bars represent SE from the means.	207
Figure 6.5	Net photosynthesis (a); instantaneous transpiration (b); stomatal conductance (c) and water use efficiency (d) of the controls, S1 and S2 leaves. Results are the means of six sample replicates. Error bars represent SE from the means.	209
Figure 6.6	Temperature of the fully expanded leaves, obtained by IR-camera. Leaf temperature is the mean of six sample replicates. Error bars represent SE from the means.	210
Figure 6.7	Total soluble sugar content (a) and total soluble protein content (b) in the controls, S1 and S2 rice shoots and roots on days 1 – 4 and days 7, 9, 11 and 14. Results are the means of three sample replicates. Error bars represent SE from the means. Means with different letters are significantly different (Tukey's test, $p < 0.05$).	211
Figure 6.8	MDA in the controls, S1 and S2 rice shoots and roots on days 1 – 4 and days 7, 9, 11 and 14 (a); carbonyl contents in the controls, S1 and S2 rice shoots on days 1 – 4 and days 7, 9, 11 and 14 (b). Results are the means of three sample replicates. Error bars represent SE from the means. Means with different letters are significantly different (Tukey's test, $p < 0.05$).	213
Figure 6.9	Hydrophilic and lipophilic antioxidant activity in rice shoots on days 1 – 4 and days 7, 9, 11 and 14. Results are the means of three sample replicates. Error bars represent SE from the means. Means with different letters are significantly different (Tukey's test, $p < 0.05$).	214

Figure 6.10	Principal Component Analysis of variability of parameters, tested for assessing growth and physiological condition of the controls and ash-treated plants (S1 and S2) (a); Vectors of the considered variables showing the correlation between them (b).	217
Figure 7.1	Conceptual cartoon summarising the main results on the potential of tephra (or ash) to form crust (Chapter 3); to serve as a niche for fungi (Chapter 4) and to affect plant growth (Chapters 5 and 6).	238
Figure 7.2	Summary of the response of the tomato and rice growth-related, physiological and biochemical parameters to ash deposition. <i>H</i> - shoot height, <i>L</i> - root length, <i>DW</i> - dry weight, <i>A</i> - net photosynthesis, F_v/F_m - maximal efficiency of <i>PS2</i> 's photochemistry in the dark-adapted state, <i>qP</i> - photochemical quenching coefficient, <i>PS2</i> - actual <i>PS2</i> efficiency, <i>NPQ</i> - non-photochemical quenching, <i>Chl a+b</i> - total chlorophyll, <i>Ccar</i> - carotenoid, <i>E</i> - transpiration, <i>g_s</i> - stomatal conductance, <i>c_i</i> - intercellular CO ₂ , <i>WUE</i> - water use efficiency, <i>T</i> - leaf temperature, <i>MDA</i> - malondialdehyde content, <i>HAA</i> , <i>LAA</i> - hydrophilic and lipophilic antioxidant activities, respectively. These parameters were monitored 8 times for 2 weeks after the 1st ash application. The results are presented for days 1-4 and 7, 9, 11 and 14. S1 and S2: a one-event scenario and a multiple-event scenario, respectively.	239
Figure B.1	Particle size distribution (wt. %) of MER , SC and EYJA ash samples.	250

Figure B.2	Cross-sectional SEM images obtained for the polished sections of the MER (A), SC (B) and EYJA (C) samples protected from raindrop impact.	254
Figure B.3	Drop effect on porosity of the MER ash deposit surface exposed to 10 mm of rainfall; 1 pixel = 0.48 μm $\times$ 0.48 μm (A); porosity of non-impacted by raindrops MER ash deposit surface exposed to 10 mm of rainfall; 1 pixel = 1.28 μm $\times$ 1.28 μm (B). Stitched 3-colour clustered image (left image in A and B); result of the porosity calculation (right image in A and B).	255
Figure B.4	Drop effect on porosity of the SC ash deposit surface exposed to 10 mm of rainfall; 1 pixel = 0.48 μm $\times$ 0.48 μm (A); porosity of non-impacted by raindrops SC ash deposit surface exposed to 10 mm of rainfall; 1 pixel = 1.28 μm $\times$ 1.28 μm (B). Stitched 3-colour clustered image (left image in A and B); result of the porosity calculation (right image in A and B).	256
Figure B.5	Drop effect on porosity of the EYJA ash deposit surface exposed to 10 mm of rainfall; 1 pixel = 0.70 μm $\times$ 0.70 μm (A); porosity of non-impacted by raindrops EYJA ash deposit surface exposed to 10 mm of rainfall; 1 pixel = 1.89 μm $\times$ 1.89 μm (B). Stitched 3-colour clustered image (left image in A and B); result of the porosity calculation (right image in A and B).	257
Figure C.1	Box plots showing differences between mean values obtained for TT, BT, T, S1 and S2 in both years (Pairwise comparisons using Wilcoxon rank sum test, $p < 0.05$).	263

Figure C.2	Box plots showing differences between mean values obtained for TT, BT, T, S1 and S2 in both years (Pairwise comparisons using Wilcoxon rank sum test, $p < 0.05$).	264
Figure C.3	In silico PCR amplification with hybridization of the primer pair ITS1 and ITS4-KY01 (a). PCR amplicon size obtained with the in silico PCR amplification of <i>Russula brevipes</i> sequence (Accession number: FJ845429) using the primer pair ITS1 and ITS4-KY01 (b).	265
Figure D.1	Particle size distributions of Pinatubo ash samples sieved through 250 μm mesh, as measured using COULTER LS 100Q laser diffractometer. .	267
Figure D.2	Photographs of leaves of control tomato plants (a); tomato plants exposed to S1 after 14 days of experiment (b); exposed to S2 after 14 days of experiment (c); controls not treated with ash (d); plants exposed to S1 (e); plants exposed to S2 (f), aerial and root parts of controls (g); aerial and root parts of plants exposed to S1 (h); aerial and root parts of plants exposed to S2 (i). . . .	269
Figure D.3	Images obtained by IR-camera and photographs of the control leaf (a); S1 leaf (b) and S2 leaf (c). . . .	270
Figure E.1	Photographs of rice exposed to S2 (a); folded leaf treated with ash after 14 days of experiment (b); twisted leaf treated with ash after 14 days of experiment (c); leaf treated with ash after 14 days of experiment (with different shades of green on the twisted part of the leaf - showed on the image with black arrow) (d); folded leaf after ash removing after 14 days of experiment (arrow shows the different shades of green colour on the folded part) (e); control rice (f); rice exposed to S1 (g); rice exposed to S2 (h).	272

Figure E.2	Images obtained by IR-camera and photographs of the control leaf (a); S1 leaf (b) and S2 leaf (c).	273
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LIST OF TABLES

Table 4.1	Chemical and microbiological properties of Mt. Etna volcanic deposits and buried soils collected in 2014 and 2015.	122
Table 4.2	Fungal species in tephra and soil, identified by DGGE.	128
Table 5.1	Photosynthesis-related parameters measured on days 4 and 14 after the first ash application. The results are the mean ± SE of six sample replicates. Chlorophyll and carotenoid contents are the mean ± SE of three sample replicates from days 1 – 4 and days 7, 9, 11 and 14.	172
Table 6.1	Photosynthesis-related parameters measured on days 4 and 14 after the first ash application. The results are the mean ± SE of six sample replicates. Chlorophyll and carotenoid contents are the mean ± SE of three sample replicates from days 1 – 4 and days 7, 9, 11 and 14.	208
Table B.1	Brief description of the ash samples used in the study. a: [1], b: [2], c: [4]	249
Table B.2	Particle size distribution (wt. %) of MER, SC and EYJA ash samples.	251
Table B.3	Chemical compositions (in mg kg ⁻¹ , except TDS in g kg ⁻¹) and pH of the ash leachates.	252

Table B.4	Results of the micro-topography and micro-morphology analyses of the MER, SC and EYJA ash deposit surfaces.	253
Table B.5	Porosity measurements: the minimum pore percentage area ($\theta_{av\ min}$) values for the MER, SC and EYJA <i>Fine-grained layer in micro-peaks (Pfgl)</i> ; <i>Fine-grained layer in micro-craters (Cfgl)</i> ; <i>Coarse-grained layer in micro-craters (cgl)</i> and the bulk layer. Results are the mean $\pm$ SE of all pixel rows in the selected regions of the stitched SEM image.	258
Table D.1	Results from the two-way ANOVA. The effects on leaf temperature, plant-related and physiological parameters from treatment (controls, S1 and S2), days of measurements (or plant age) and the interaction between treatment and plant age. ns – nonsignificant. The data for chlorophyll fluorescence was excluded from the analysis, since the results were presented only for 2 days of measurements.	268
Table E.1	Results from the two-way ANOVA. The effects on leaf temperature, plant-related and physiological parameters from treatment (controls, S1 and S2), days of measurements (or plant age) and the interaction between treatment and plant age. ns – nonsignificant. The data for chlorophyll fluorescence was excluded from the analysis, since the results were presented only for 2 days of measurements.	271

LISTINGS

Listing B.1 Python script for porosity measurements [259](#)

ACRONYMS

TRB	Total reserve in bases
DGGE	Denaturing gradient gel electrophoresis analysis
PCR	Polymerase chain reaction
TAS	Total alkali-silica
BLS	Bivariate least square regression
TOM	Total organic matter
TOC	Total organic carbon
TN	Total nitrogen
WC	Water content
FB	Fungal biomass
DNA	Deoxyribonucleic acid
DW	Dry weight
ITS	Internal transcribed spacer
LI	Loss on ignition

WGA	Wheat germ agglutinin
FITC	Fluorescein isothiocyanate
HPLC	High-performance liquid chromatography
ECM	Ectomycorrhizal
C/N	The ratio of total organic carbon to total nitrogen
PVC	Polyvinyl chloride
EYJA	Eyjafjallajökull volcano, Iceland
SC	San Cristobal, Nicaragua
MER	Mt. Merapi, Indonesia
SEM	Scanning Electron Microscopy
MDA	Malondialdehyde
WUE	Water use efficiency
HAA	Hydrophilic antioxidant activity
LAA	Lipophilic antioxidant activity
A	Net photosynthesis
c_i	Intercellular CO ₂ concentration
E	Instantaneous transpiration
T	Leaf temperature
g_s	Stomatal conductance
DW _s	Shoot dry weight
DW _r	Root dry weight

WC_s	Shoot water content
WC_r	Root water content
F_v/F_m	The maximal efficiency of <i>PS2</i> 's photochemistry in the dark-adapted state
H	Shoot height
L	Root length
(chl a + chl b)	Total chlorophyll
Ccar	Carotenoid
qP	Photochemical quenching coefficient
NPQ	Non-photochemical quenching
ROS	Reactive oxygen species
R_{cp}	Maximum crater to peak height
P_c	Micro-peak counts
C_c	Micro-crater counts
Tfgl	Thickness of fine-grained surface layer
Tcgl	Thickness of the coarse-grained surface layer
Cfgl	Fine-grained layer in micro-craters
Pfgl	Fine-grained layer in micro-peaks
cgl	Coarse-grained layer in micro-craters

Part I

INTRODUCTION

GENERAL INTRODUCTION

1.1 CONTEXT

Nowadays, approximately 800 million people inhabit areas within a radius of less than 100 km from a volcano ([32]). The reason driving many populations to settle in the proximity of active volcanoes is the high fertility and therefore, high agricultural potential, of volcanic soils that result from the devastating emissions of pyroclastic materials, their deposition and weathering in the exposed areas ([5]). Volcanic soils cover more than 124 million hectares on the Earth's surface, which is equivalent to 1% of soils in the world ([35]).

However, besides the benefits that people have from the use of volcanic soils, a large proportion of the world's population is exposed to volcanic hazards. According to the Smithsonian Institution (2013), there are currently about 1551 volcanoes on Earth, 866 of which have been active in the last 10000 years ([32]). About 600 of them are still active producing about 63 eruptions each year and 86 countries of the world host one or several active volcanoes ([32]). Volcanic eruptions are one of the most dangerous phenomena on Earth. Throughout time they have impacted human society and the natural environment on various scales. Volcanic eruptions can lead to the loss of life and livelihoods in communities living in a close proximity to the volcanoes, damage critical infrastructure and agricultural systems, displace populations, disrupt business and add stress to already fragile environments (e.g., [29]; [8]). At their worst, volcanic eruptions have caused some of history's most catastrophic disasters, modifying global climate and precipitating the demise of several of the world's major civiliza-

tions.

The hazards from volcanic eruptions can be from pyroclastic density currents, lava flows, lahars, volcanic gas emissions and tephra falls ([40], Part 7). Explosive eruptions typically inject tephra high in the atmosphere, and the most powerful ones generate ash clouds (ash is tephra with particle size less than 2 mm) that can spread around the globe ([40], Part 4, Chapter 25). Tephra fall is the most frequent and far-reaching volcanic hazard, and therefore one of the most threatening to society and surrounding environment ([32]; [8]). Tephra emissions spaced over periods of varying length (from a few weeks to several years) pose major problems to agricultural ([43], [44]; [11]) and natural ecosystems (e. g., [24]; [23]; [1]; [2]; [3]; [17]). Tephra fall disturbances of the surrounding environment may occur due to burial, canopy abrasion and loading, chemical toxicity and asphyxiation of animals ([40], Part 9, Chapter 73) etc. Nonetheless, areas affected by tephra often become a source of livelihood over the long-term scale as weathering of their deposits typically produces soils that are among the most fertile in the world ([39]). Volcanic eruptions also provide other long-term benefits to society, such as geothermal resources, the utilisation of volcanic materials (e. g., basalt hammers and pumice in soap), many precious metal and gemstone deposits ([16]; [18]), tourism together with archaeological and artistic gains ([40], Part 9; [29]; [27]; [6]) and beneficial landscapes (e. g., auditorium Auditorio Jameos del Agua on Lanzarote, Canary Islands, Spain) ([27]).

At the same time, the intermittent disturbance of ecosystems by tephra emissions is often inevitable in the agriculturally attractive areas. Previous studies focused mostly on the impact of chemical compounds released from tephra on soil, vegetation, water, animal and human health ([12]; [14]; [13]; [25]; [46]; [45]; [5]). For instance, the presence of toxic elements, such as fluorine, in tephra deposits may cause fluorosis in grazing animals ([14]; [13]; [21]; [25]). Oxidation of sulphur minerals, such as elemental sulphur and pyrite, sometimes associated

with tephra, acts as a source of acidity. When tephra is deposited on plants, this reaction may damage foliage and potentially reduce plant growth ([30]; [5] and references therein). On soils, sulphur-rich tephra may impact soil pH ([14]). Such chemical effects typically originate from tephra containing secondary hydrothermal minerals eroded from the volcanic conduit during so-called phreatomagmatic eruptions, or in the early phase of a magmatic eruption ([46]). However, they are not widespread and are not representative of the majority of tephra from explosive eruptions. The common assumption that tephra impacts on ecosystems are largely chemically mediated is probably not accurate and it certainly does not apply to all situations (e. g., [45]).

Besides the potential but relatively local chemical effects of tephra, there is strong evidence for physical impacts of tephra deposition on vegetation and soils. These include burial, breakage, abrasion, loading and smothering of vegetation, and changes in soil physical properties, for instance, infiltration rate (e. g., [15], Chapter 4; [2]; [10]; [43], [26]). The economical consequences of tephra deposition on agricultural systems may be severe and long-lasting (e. g., [10]; [36]; [43]). Since the agricultural benefits obtained from cultivating volcanic soils attract a large population in active volcanic regions, efforts are being made to better inform the communities about the potential hazards and risks posed by tephra fall to agriculture ([41]). This can contribute to the development of good management practices before and after a tephra-producing eruption ([11]).

A research aimed at unravelling some of the mechanisms driving the complex interactions between tephra, vegetation and soil should improve our capacity to predict the physical impacts of tephra, with various characteristics, on soil and vegetation. Obviously, this is not an easy task since various factors can modulate the type and magnitude of impacts. These include tephra properties, deposit thickness and frequency, weather conditions, ecosystem characteristics, time of the year,

etc.

In this PhD thesis, I focus on three different themes:

- surface crusting of ash deposit (particle size <2 mm);
- early stage colonization of tephra (particle size >2 mm);
- effect of an ash deposit on the growth and physiology of crop plants.

The justification for studying these themes, which pertain to different scientific disciplines, is largely rooted in the overarching objectives of the EU-FP7 ITN project Vertigo¹, which supported my research (A.1). The choice was also partly motivated by opportunities and constraints that appeared during this PhD research.

A common phenomenon that may temporarily affect a soil covered by ash is the formation of a crust at the surface of the deposit. Ash crusting alters the soil-atmosphere interface. It has been shown to impede the re-emergence of vegetation and to lead to decreased plant growth ([1]). Further, a crusted ash deposit reduces water infiltration which in turn, alters the soil water balance and increases runoff ([34]; [31]). There is new evidence that this contributes to enhance lahar (debris flow) initiation in ash-impacted areas ([26]). While surface crusting of agricultural soil has been well studied (e.g., [42]; [9]), no effort has been made to understand the mechanisms of crust formation in an ash deposit on soil. This knowledge is needed in order to improve our understanding of how ash deposited on soils may alter their properties and hence, functioning.

A tephra deposit on soil can modify the activity of below-ground microorganisms ([24]). This is due largely to the new environmental

¹ Volcanic ash: Field, experimental and numerical investigations of processes during its life cycle; <https://vertigo-itn.de/>

conditions imparted by the volcanic deposit properties (i. e. chemical composition, porosity, etc.). Notably, fresh tephra is devoid of the key nutrient nitrogen, and phosphorous may also in some cases be limiting ([28]). Nonetheless, tephra often represents a substrate that microorganisms, and notably fungi, can colonize ([20]; [22]; [37]). Knowledge on fungal community distribution, diversity, composition and origin in freshly-deposited tephra should provide insights into the processes of community evolution and interaction, while also providing information on soil formation after a natural disturbance.

The detrimental effect of tephra deposition on vegetation has long been recognised. The vast majority of knowledge on this subject comes from field observations undertaken after the eruption of Mt. St. Helens in the USA in 1980 (e. g., [4]; [7]; [33]; [38]; [19]). Besides direct mechanical damages linked to heavy tephra loading on plants, a layer of particles on foliage may have detrimental morphological and physiological effects (e. g., [24]; [38]; [23]). In turn, these may lead to significant reduction in plant growth and thus, yield. Tephra-induced impeded photosynthetic activity is suspected to occur but it has not been proven. It is also not clear if a given amount of tephra deposited by a single event will generate the same impact compared to when it is deposited via multiple events spaced in time.

Here, I apply a combination of field and laboratory methods and techniques, borrowed from several disciplines such as microbiology, biochemistry and soil and plant sciences, to address three research questions:

1. What are the mechanisms involved in the formation of a crust at the surface of an ash deposit on soil?
2. Does young tephra serve as an ecological niche for fungi and if so, how does the colonization operate?

3. Does an ash deposit on crop plants affect growth and leaf physiology?

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2.1 OVERVIEW OF A VOLCANIC ERUPTION

Volcanoes can be different in morphology, evolution, eruptive styles, and behaviours dictated by a variety of tectonic settings, magma production rates and compositions, and eruption conditions that they represent ([15]). Explosive volcanism is associated with the most dangerous type of eruption. These eruptions typically involve silica-rich magmas (dacitic and rhyolitic), and therefore generally more viscous than mafic magmas. During the ascent of the magma towards the surface the solubility of the volatile components (water, carbon dioxide, sulfur dioxide) present in the magma decreases. The volatile components separate from the solution to form bubbles, which as a result increases magma viscosity and generates high internal pressures within the gas bubbles ([70]) (Fig. 2.1). The resulting overpressure in the magma will eventually lead to the sudden bursting of gas bubbles and fragmentation of the magma. Magma fragmentation lowers the viscosity and bulk density of the magma, causing the gas-particle mixture, which is typically 5 to 10 times denser than air, to accelerate to very high speeds (hundreds of meters per second) and to emerge as a momentum-dominated jet into the atmosphere up to a few kilometres ([15]). When the jet is discharged, the interaction of the emerged particles, hot gases from the magma, pieces of the volcanic edifice, referred to as wall rock lithics form an eruptive column, and air causes the formation of high eruption plumes that produce tephra (particles generated by magma fragmentation) fallout ([70]; [15]). Heating of the air leads to a decrease of the mixture density, which becomes lower than the surrounding environment. As a result, the jet transforms into a buoyant plume (Fig. 2.1, convective phase) forming a high eruptive

column in the atmosphere. The plume finally reaches the level of neutral buoyancy spreading laterally (Fig. 2.1, umbrella region) and transporting the tephra over great distances. Sedimentation of tephra particles out of the plume produces tephra fallout, which may blanket terrestrial ecosystems (Fig. 2.2).

2.2 PHYSICAL AND CHEMICAL PROPERTIES OF TEPHRA

Texturally tephra particles are divided into several classes: blocks and bombs (64 mm - 256 mm), lapilli (64 mm - 2 mm) and volcanic ash (<2 mm) ([33]). Volcanic ash refers to very fine, fine and coarse particles with diameter <63 μm , between 63 and 100 μm and between 100 and 2000 μm , respectively ([8]).

Sedimentation of tephra particles strongly depends on their residence time in the plume, on their terminal fall velocities and their ability to form aggregates while airborne. The residence time of particles in an eruption plume is inversely proportional to its terminal fall velocity, which scales with clast diameter and density. Thus, the denser minerals present in tephra will be deposited faster and closer to the vent than the glassy component of tephra. Larger particles sediment closer to the vent, while finer particles are transported over longer distances. Therefore, the average size of the deposited particles decreases away from the vent. Particles may aggregate within the volcanic plume and in the tephra cloud. If particles form aggregates via collision and binding, the dispersion and sedimentation of tephra particles will be affected. Tephra aggregates will settle out of the plume faster, thereby decreasing the residence time of the tephra particles in the atmosphere. As a result of tephra aggregation, secondary maxima of particle accumulation may occur locally increasing deposition rate and altering patterns of tephra thinning with distance from the vent ([70]; Part 4, Chapters 33, 34).

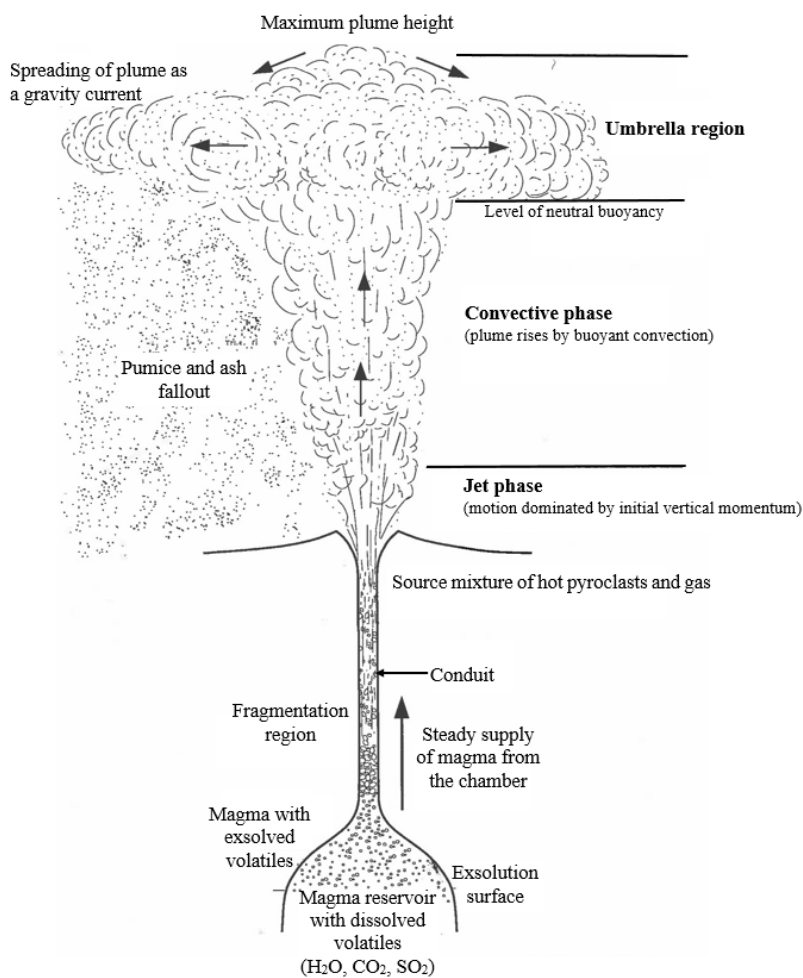


Figure 2.1: Schematic representation of ascent of the gas-particle mixture in the volcanic plume to the height of neutral buoyancy and dispersion of the volcanic cloud ([70]).

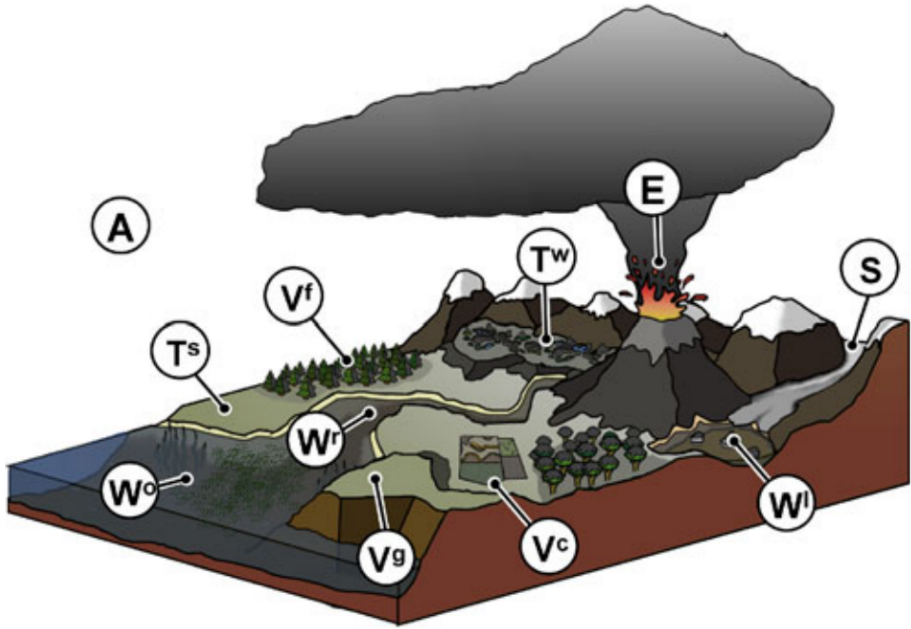


Figure 2.2: Cartoon diagram illustrating the receiving environments of tephra that undergone transition through the volcanic plume and cloud (E) before environmental interaction occurs. Tephra is then emitted into the atmosphere (A). Deposition will then carry the particles onto vegetation surfaces (V), such as forests (Vf), cropland (Vc) and grasslands (Vg). Tephra may also be deposited onto land surfaces (T), such as soil (Ts), wetlands (Tw) or onto snow or ice (S). Particles falling into the aquatic system (W) may interact with the ocean and ocean sediments (Wo), lakes and lake sediments (Wl) and river and river beds (Wr) ([8]).

Tephra can have very different bulk chemical compositions depending on the nature of the rocks and the type of magma from which these rocks originate. The most general classification of volcanic rocks is based on their SiO_2 content. Volcanic rocks have basic or mafic (45-52 wt% SiO_2), ultrabasic or ultramafic (<45 wt% SiO_2), intermediate (52-66 wt% SiO_2) or acidic or silicic (> 66 wt% SiO_2) compositions ([87]). As the composition of volcanic rocks varies considerably, the standard variation Total Alkali Silica (TAS) diagram is being used to define rock names ([51]). The diagram represents a graph of total alkalis ($\text{Na}_2\text{O} + \text{K}_2\text{O}$) against SiO_2 content (Fig. 2.3).

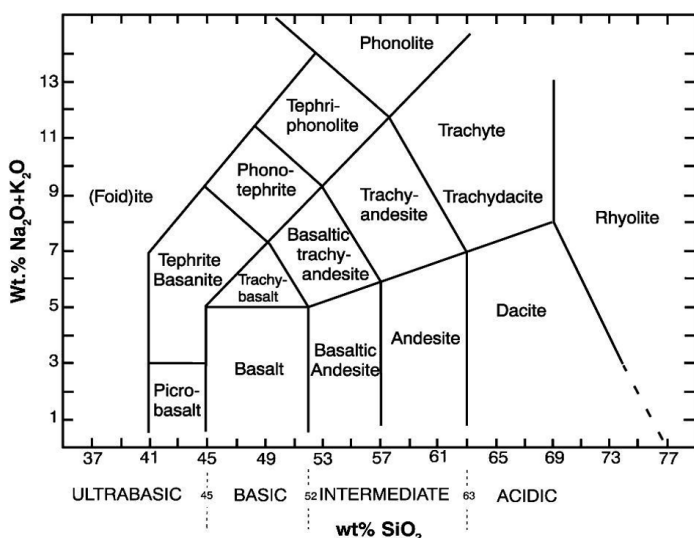


Figure 2.3: The chemical classification of the volcanic rocks based on total alkalis versus silica (TAS). Superimposed are the boundaries that delineate different rock types according to the International Union of Geological Sciences (IUGS) ([51]) with the names of volcanic and selected plutonic rocks types. Taken from [51].

The crystallinity of tephra also depends on magma types and varies across eruptions. At the same time, as magma rises to the surface, some elements (e. g., Fe, Mg, Ca) dissolved in the melt may be preferentially incorporated into crystals that form as magma pressure and temperature decrease. Therefore, tephra generated upon magma fragmentation consists of both glass and crystal (mineral) components in various proportions (e. g., quartz, feldspars, plagioclases, pyroxenes, olivines, Fe and Fe/Ti oxides) ([38]). Their presence in tephra may also result from contamination by lithics from previous eruptions([70], Part 1). These may consist of hydrothermal minerals (e. g., sulphate and halide salts) ([28]), such as anhydrite (CaSO_4), fluorite (CaF_2), fluoroapatite ($\text{Ca}_5(\text{PO}_4)_3\text{F}$), elemental sulfur (S) and sulfides (Na_2S) (e. g., [20]).

The composition and viscosity of the magma also influence the physical properties of tephra, such as density ([68]), colour, and therefore the refractive and reflective properties ([63]), and the shape of the particles (e. g., spherical, elongate, blocky, bubble wall shards) (Fig. 2.4) ([38]). Finally, the surface reactivity of tephra particles is governed by the specific surface area (defined as an area per unit mass of tephra, m^2/g) and the tephra surface chemical composition. The specific surface area is inversely proportional to the tephra size: the finer the particles, the larger the specific surface area.

Large explosive eruptions produce a larger amount of very fine tephra than small explosive eruptions ([8]). The tephra surface chemical composition comes from the magmatic volatile species present in the volcanic plume. For example, the elements that can be present on the tephra surface as salts are sulfur, chlorine and sometimes fluorine, as well as sulphate salts (e. g., CaSO_4 , Na_2SO_4) and halides (e. g., NaCl, KCl, CaCl_2) ([27]). Depending on the solubility of the sulphate and halide salts present on tephra surfaces, the tephra deposit exposed to water (rain, surface waters, etc.) may release various amounts of cations and anions in the environment. In turn, these may positively or negatively impact biotic and abiotic processes ([20]; [8]).

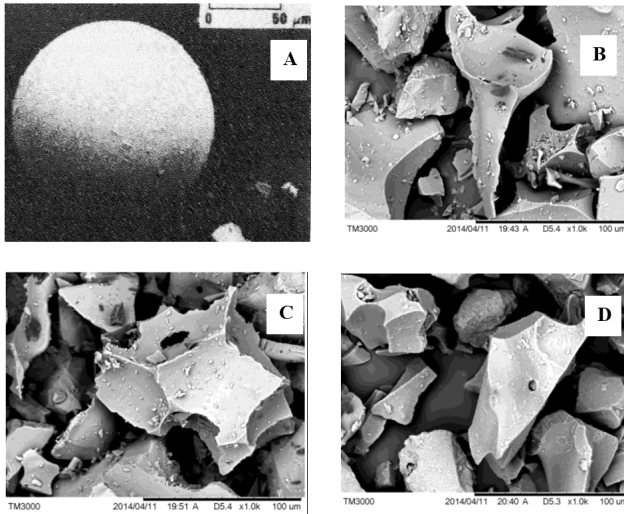


Figure 2.4: Scanning electron microscopy images of the tephra particles with different shapes: spherical tephra particle (a) ([38]); elongated tephra particle (b), tephra particle with sharded bubble walls (c), blocky tephra particle (d) ([47]).

2.3 ENVIRONMENTAL EFFECTS OF TEPHRA

Tephra emissions occur frequently and can influence various terrestrial and aquatic environments on different temporal and spatial scales ([8]). Depending on their magnitude, volcanic eruptions can affect large areas and cause significant damage to natural and agricultural ecosystems (Fig. 2.5). However, the impact of smaller tephra emissions may also be dramatic. Though often neglected, small releases of tephra that are associated with weak or moderate but repeated volcanic activity generate enormous problems for local communities. These communities are often represented by people who rely heavily on agriculture ([55]). Therefore, among the tephra receiving environments, vegetation and soil systems are of high importance as their damage may result in

lower agricultural potential of the affected areas due to the decreased net productivity, loss of crops, water contamination etc. ([5]; [8] and references therein).

For example, the ash deposits after eruption of Mt St. Helens in 1980 diminished the production of crop, which cost was estimated to be 100 million dollars in 1980 or 7% of the normal crop value. The loss of alfalfa production was estimated to be 35 million dollars, while the loss of apple production was about 15 million dollars ([16]). The fertility of soils in the areas close to Tungurahua volcano in Ecuador encouraged populations to settle in the vicinity of the volcano ([6]). However, one of the recent eruptions of Tungurahua in 2006, affected about 15000 farmers, or 50000 hectares of agricultural areas where corn, potatoes and pastures (for livestock) were lost due to the tephra deposition. In 2012, tephra emissions from the frequent volcano explosions also resulted in the loss of thousands of hectares of crops (maize, potatoes, beans, pastures) and ten thousand dairy cows, which decreased milk production by 50 %. In 2014, after a strong eruption, a large deposit of tephra affected maize seedlings, "tomatoes of árbol" (Ecuadorian fruit that resembles an elongated tomato), pasture and chickens. The remobilisation of the ash after the eruption of Mt. Hudson, southern Patagonia, South America in 1991 also severely damaged the agricultural economy of the region ([84]). Volcanic material was deposited over 100000 km², with thickness of over 1000 mm measured in proximal areas ([18]). Although there was no direct damage of the seasonal crops by ash fall, as eruption happened in winter and most of the plants were not growing, the long-term disturbance of horticultural plants was noticeable. The farmers faced cultivation difficulties during 17 years after the tephra deposition. The socio-economic crisis can therefore be enormous due to tephra disposition ([6]; [36]).

The intensity and duration of the environmental impacts of tephra are governed by a combination of factors, such as time of the year (e. g., harvest season or planting season), the residence time of tephra de-

posit in terrestrial ecosystems, the physico-chemical characteristics of tephra deposits (e. g., deposit thickness, chemical reactivity of tephra) and the environmental receptor characteristics ([8]; [18]).

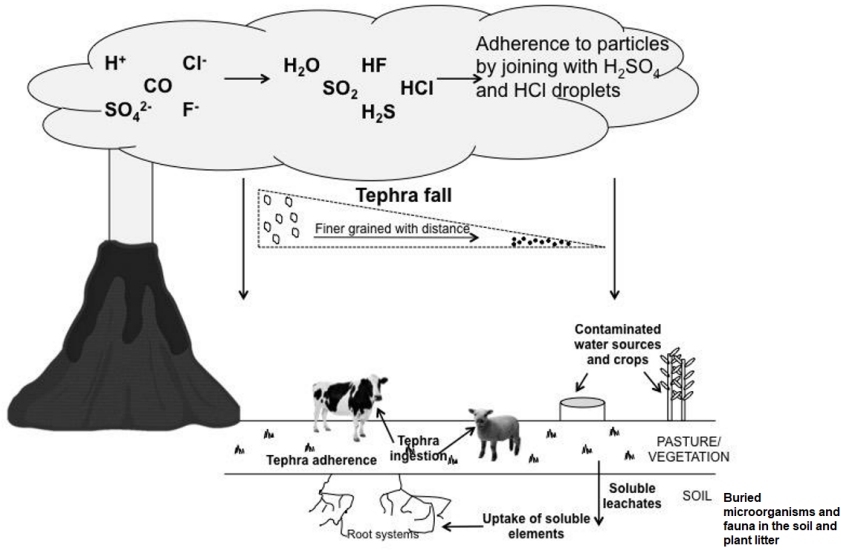


Figure 2.5: Simplified cartoon showing the tephra fall depositions and compartments that may be affected by tephra, including animals, vegetation, soil and microorganisms. Adapted from [17].

2.3.1 Residence time of tephra on soil

The residence time of freshly-deposited tephra, not incorporated in soil by vegetation, animals or human activity, is dictated by wind, water erosion and rainfall events ([8]). Wind erosion can appear in three forms: creep (rolling), saltation and suspension (Fig. 2.6). Most likely, the particles with lower density below 0.1 mm in diameter are redistributed on the surface by suspension caused by particles subjected to saltation ([71]). Particles with diameter range between 0.1-0.5 mm are

redistributed along the surface by saltation. Particles above 0.5 mm in diameter are moved by creep. The moved particles in turn displace the finer ones. This process starts once wind threshold velocity is reached ([71]).

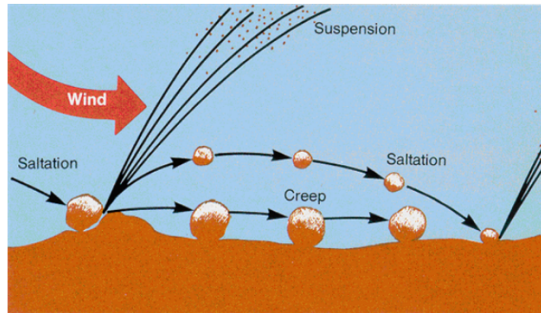


Figure 2.6: Three forms of wind erosion: creep (rolling) of particles with diameter above 0.5 mm; saltation (bouncing) of particles with diameter range between 0.01-0.5 mm and suspension of eroded particles by particles subjected to saltation. Image is taken from www.milford.nserl.purdue.edu.

Aeolian erosion and resuspension of tephra can last for a short time (weeks to months) (e. g., [82]) or for longer period (months to years) after the eruption ([46]; [42]; [84]) and can result in the altered landscape features. In general it is stronger in open dry areas ([84]) and weaker in areas covered by vegetation ([84]; [79]).

Tephra removal by water can occur as sheet, rill, gully and ravine erosion (Fig. 2.7). Higher erosional hazards occur on steeper slopes. The sheet water erosion affects low-angle slopes, while rill, gully and ravine erosion is typical for steeper slopes ([75]). Sometimes, debris flows are generated through mobilisation of tephra during intensive rainfalls. This can be destructive leading to the flooding of areas in the

proximity of volcano ([43]).

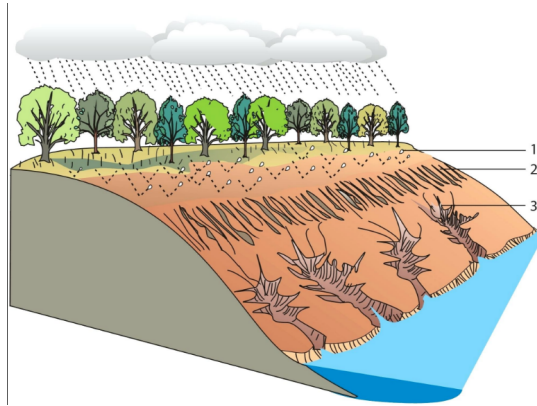


Figure 2.7: Diagram representing the forms of water erosion: sheet erosion (1); rill erosion (2); gully and ravine erosions (3). Diagram is taken from www.next-book.co.

Rainfall events directly after tephra deposition can lead to tephra cementation ([16]; [53]). However, the processes involved in tephra cementation are not well understood.

2.3.2 General physical effects

As illustrated in Figure 2.8, tephra on soils may induce a variety of effects. The direct effect of tephra deposition on soil can manifest in a soil albedo change. The tephra colour dictates the thermal alteration of the underlying soil. The dark-coloured tephra typically corresponding to basaltic composition decreases soil albedo, potentially increasing soil temperature, which may in turn cause heat stress in roots ([72]). Conversely, light-coloured tephra, typically corresponding to more silicic composition, increases soil albedo, which may lead to lower soil temperatures ([11]; [16]). The high albedo of more silicic tephra was shown

to decrease soil temperature by 6-10 °C after Mt. St. Helens eruption ([16]). However, the tephra colour gradation based on the chemical composition of the tephra is inaccurate as tephra colour can change depending of the grain size (e. g., [36], Fig. 2.9).

A tephra deposit on soil can affect water and gas exchanges between soil and atmosphere. For instance, tephra can enhance water conservation in soils, i.e. become an effective material for soil moisture conservation - mulch, due to its low retention capacity and high hydraulic conductivity, which facilitates water infiltration and decreases evaporation losses ([78]). As a result, more water becomes available for plants that leads to an increase in average crop production ([76]; [77]; [16]; [84]). The mulch efficiency makes irrigation-free agriculture possible, which is particularly beneficial in regions with low precipitation amounts. The use of tephra as a mulch has even been proposed in a commercial sector ([78]; [76]). However, it is important that the tephra depth is not high enough to avoid suppression of the positive mulching effect by completely terminating the soil processes.

The extent of the tephra impacts on water and gas exchanges between soil and atmosphere is mainly attributed to the tephra particle size, pore size distribution of the tephra deposit, tephra porosity, deposit thickness and hydraulic properties ([8]; [78]). A tephra layer with high hydraulic resistance (thick deposit, low hydraulic conductivity) will hamper infiltration, possibly evaporation and aeration if deposited on top of a permeable soil (e. g., [53]; [40]; [31]; [41]). The finer the tephra particle size, the greater the loss of infiltration capacity ([16]; [53]; [39]; [60]).

Tephra cementation can further impact water infiltration rate ([16]; [53]). Cementation has been associated mostly with fine-grained tephra ([57]; [45]), but the mechanisms of cementation are not fully understood. It has been suggested that the filling of pores with fine particles mobilized by raindrop impact is an important process that leads to di-

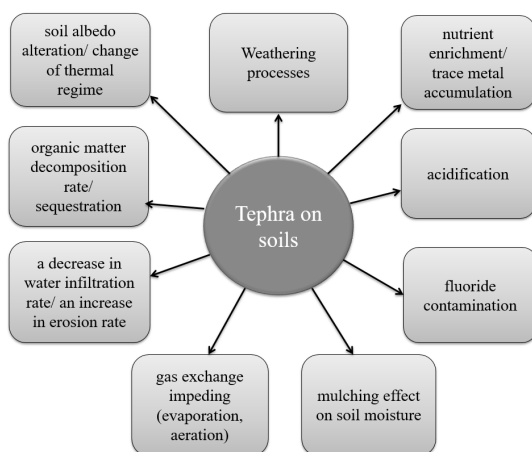


Figure 2.8: Potential impacts of tephra on soils.

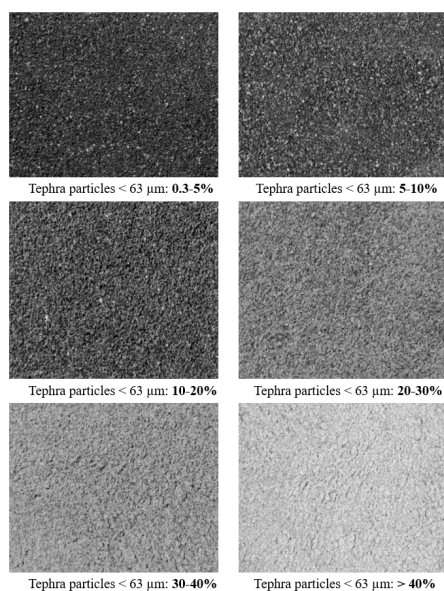


Figure 2.9: Photographs of tephra from the eruption of Tungurahua volcano showing the colour differences with respect to the fine content (<63 μm) of the tephra ([36]).

minished tephra permeability ([16]; [64]; [39]). Tephra compaction as a result of raindrop impact, and porosity decrease over time with wetting and drying cycles may also lead to lower hydraulic conductivity ([64]). As a result, crusts formed in tephra deposits may diminish water storage in the underlying soil, cause runoff and hence erosion ([57], [45]).

2.3.3 *Chemical effects*

Fresh tephra deposits on soil may release acidic compounds upon exposure to rainwater. This may result in a transient drop in pH of the soil ([22]; [21]). Tephra leaching could be accompanied by the release of toxic concentrations of Al in the soil solution along with a loss of some soil exchangeable cations (Ca, Mg, K and Na) ([22]). However, young volcanic soils have a good capacity to buffer and neutralise acids via ion exchanges and weathering processes ([22]; [26]). This ability may prevent the long-term volcanic soil acidification initiated by the acid release from the freshly deposited tephra ([8]) (Fig. 2.8).

Another potential environmental impact of tephra on soils is contamination with fluoride. It is of significant importance since overexposure to fluoride may lead to human and animal fluorosis (Fig. 2.5) ([21]; [20]; [34]; [44]; [83]). Fluoride in soils has a low mobility, usually it is strongly retained in the surface soil layers and, therefore, it usually does not present a danger for the growth of most plants ([13]) (Fig. 2.8).

A tephra deposit on soil can also bring positive effects to nutrient-poor soils by releasing key agronomically useful elements such as P, S, Ca, Na, K, Mg, Se and in small amounts B, Co, Cu and Zn (Fig. 2.5) ([21]). The nutrient release occurs when deposited tephra liberates cations via silicate dissolution and/or surface exchange with proton donors - water or acids, coming from either eruption plume (sulphuric acids H_2SO_4 , hydrochloric acids HCl, fluoric acids HF), or from biota

(carbonic and organic acids) ([24]) (Figs. 2.5 and 2.8). Unfortunately, the content of most elements in tephra is so low, that in order to gain essential benefits, required tephra amounts would be prohibitive to plant growth ([8]). However, low nutrient addition from tephra is not always the case. For instance, it has been shown that sulphur may be released from tephra deposit after the Ruapehu eruption in useful amounts ([21]). Organic matter from vegetation and soil is not usually present in tephra leachates, unless it volatilises during the eruption and entrains in the hot volcanic plume ([59]).

2.3.4 *Biological effects*

The deposition of tephra on terrestrial ecosystems may modify fundamental interconnected soil processes, such as organic matter decomposition and mineral weathering by microorganisms. The freshly-deposited volcanic material is devoid of organic matter and organic nitrogen and therefore may limit microbial colonization and activity ([49]). Tephra deposition may alter decomposition of buried organic matter due to the changes in soil and plant litter microbial and faunal activity (Fig. 2.5) ([25], Part 4, Chapter 16; [9]). [25] predicted that volcanic disturbance of the exposed zones may inhibit the decomposition rate of buried organic matter by decreasing or preventing the activities of decomposer organisms, such as, for example, microarthropods, bacteria and fungi. Decreased organic matter decomposition rates were found in the areas covered with 180 mm of tephra from Mt. St. Helens eruption in 1980 ([80]). Similarly, it was assumed that the decreased concentrations of dissolved organic carbon in soils artificially treated with 150 mm of tephra from the same eruption were due to a decrease in the decomposition rate of organic matter ([23]). However, this alteration of organic matter decomposition in buried soils may depend on the tephra deposit depth, as an opposite trend in decomposition rate was observed for soils buried under 50 mm of tephra ([80]).

On the other hand, microorganisms in the buried soil and particularly in plant litter may positively respond to the overlying tephra deposit, which becomes a newly formed low-nutrient environment. The reaction of microorganisms on the potentially scarce availability of nutrients in the tephra can be expressed through the considerable increase in synthesis of extracellular enzymes that could mobilize resources of complex organic substrates ([9]). The stimulated microbial activity in the tephra-buried organic matter may potentially alter the biogenic emission of CO_2 ([40]).

The microorganism activity in the tephra-buried organic-rich soil may contribute not only to alteration of CO_2 exchanges between soil and atmosphere, but also to tephra weathering rate. The biogenic CO_2 (e. g., from microbial, root respiration), transported to the tephra layer from the organic-rich buried soil layer, hydrates to produce carbonic acid, which releases protons. These protons exchange for base cations. This results in the downward migration of the base cations with bicarbonate from tephra (pH 6-7) to soil (pH = 4) layers, where bicarbonate protonates to H_2CO_3 . In the organic-rich soil layer H_2CO_3 in the soil solution reequilibrates with the high pCO_2 . This carbon migrates upwards as CO_2 gas and participates in another weathering cycle ([24], Fig. 2.10).

Tephra deposition on soil may serve as a potential substrate for microorganisms, particularly fungi. These can colonize the deposit in order to obtain nutrients, thereby potentially contributing to biological weathering of the substrate ([12]; [62]). Among the soil microorganisms, fungi play an important role in the bio-weathering of rock-forming minerals, as they are able:

1. to concentrate energy and biomass on specific weathering locations (through carbon translocation via their mycelia), avoiding the need to rely on diffusion through bulk soil solution;

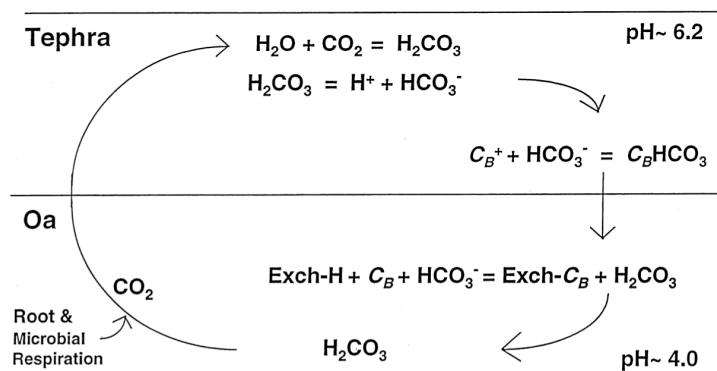


Figure 2.10: Schematic representation of the $CO_2/H_2CO_3/HCO_3^-$ - weathering/transport cycle, occurring between the tephra and Oa (organic-rich) horizon of the buried soil. C_B is a base cation. ([24]).

2. to form symbiosis with plant-roots (mycorrhiza) or algae (lichens), receiving a significant part of the carbon compounds generated by their phototrophic partners ([74]);
3. to reach distant location, not available for bacteria, through the hyphae proliferation ([50]).

Mineral degradation by fungi can be performed through biochemical and biophysical processes (mineral colonization, fungal exudation, cell wall-mineral surface interactions) ([74]).

However, tephra may also indirectly suppress mycorrhizal fungi activity through the impact on plant roots. Tephra on soil or on foliage may lead to increased mortality of fine roots. If these roots are involved in symbiosis with mycorrhizal fungi, this may reduce mycorrhizal root biomass ([40]).

2.4 TEPHRA DEPOSITION ON VEGETATION

Tephra deposition affects vegetation through both physical and chemical processes ([8]).

2.4.1 *Residence time on vegetation*

The characteristics of the leaf surface partly dictate the residence time of tephra on vegetation. Leaf surface roughness may decrease erosion of tephra by wind and water, therefore increasing the tephra retention time on the plant ([8]). For example, smooth, waxy leaves hold tephra particles for shorter periods compared to hair-covered leaves ([16]). The deposit thickness influences the residence time of tephra on plants. Heavy tephra deposit may cause leaf abscission, decreasing tephra loading time on plants. The residence time of tephra on leaf can increase if tephra is moistened because of potential cementation of tephra particles ([65]). A layer of cemented tephra may remain present up to several months after deposition, as was the case with conifers affected by tephra during the eruption of Mt. St. Helens ([5]).

2.4.2 *Physical effects on vegetation*

The consequences of tephra deposition on foliage for vegetation can be different depending on the amount of tephra and vegetation type. The effects of tephra are proportional to the thickness of the layer covering the plants and may vary according to the size distribution of tephra particles and the frequency at which new tephra is deposited ([5]; [56]; [65]). Trees or taller plants not buried by the tephra deposit of about 1-2 m thick may survive (Fig. 2.11a) ([32]; [35]), but most likely will suffer from physical damage of leaves, stems and branches (Fig. 2.11b, 2.12a) ([5]). Conversely, most short herbaceous species, shrubs, mosses, lichens and algae can be damaged by much lower tephra amounts (20-150 mm thick deposits), being partly or completely buried and not able

to recover temporarily or at all (Fig. 2.12b) ([4]; [3]; [37]). Cementation of the surface tephra layer may serve as a barrier for seed emergence ([85]). However, formation of cracks and erosion channels in the tephra deposits may be beneficial for plant reappearance (Fig. 2.13a) ([2]; [5]).

The morphology of the plant species exposed to tephra fall is an additional factor that influences the intensity of the observed damage. Pubescent plants will more easily retain tephra particles through their trichomes, and small fragile plants will more easily tilt under the heavy tephra weight ([16]; [56]; [73]). If buried by tephra, plants may develop survival mechanisms through morphological transformation ([56]) such as the emergence of a second panicle, the formation of thinner shoots capable of crossing the tephra layer, etc. ([56]; [41]).

Tephra deposition on leaves may result in the localised death of leaf tissues (known as necrosis) caused by the abrasiveness of tephra to the plants. The physical damage of the leaf by wind-remobilised tephra may last during a long period after the eruption ([84]; [85]). It was reported that for vegetation (*Populus*) growing further than 100 km from Mt. St. Helens during periods of strong winds, tephra abrasion caused premature foliar abscission ([10]). Tephra can also have an indirect effect on tree growth. Additionally to needle abscission, it was reported that tephra deposition on trees after the eruption of Mt. St. Helens caused a decrease in diameter of the trunks and their elongation ([5]).

2.4.3 *Chemical effects on vegetation*

Tephra on foliage when in contact with water may damage plants due to the release of soluble compounds, such as for example halide salts. It has been suspected that ionic salts, leached from tephra, caused the damage of the blueberries after the eruption of Mt. St. Helens ([16]. Halide salts ([61]), including fluoride compounds ([83]) may lead to the chemical wounding of the foliage. Besides, tephra can contain pyrite

(FeS₂), which oxidises to form sulphuric acid in the presence of water. The release of sulphuric acid in turn may attack the leaf cuticle and cause necrosis (Fig. 2.13b). A reduction in plant growth can also be observed ([52]; [58]; [67]). In addition, acids in contact with leaf can lead to the loss of nutrients (e. g., calcium, magnesium, potassium) due to ionic exchange ([1]). The intensity of the observed impacts depends on the sensitivity of each species to these acid compounds and salts, the composition of tephra and the contact time with water. These chemical effects can potentially hinder plant photosynthesis as ([54]; [14])

1. damaged cuticle cannot protect the mesophyll layer containing the photosynthetic cells that receive and process carbon dioxide to produce glucose;
2. losses of nutrients affect important processes such as enzyme activity, chlorophyll production, control of stomatal opening/closing).

Finally, tephra deposition on soil may indirectly affect vegetation via releases of elements and consequent plant uptake (Fig. 2.8) ([19]; [21]). In general, any soil deficiencies or toxicity will potentially affect plant health. The thickness of the tephra deposit partly dictates the magnitude of the impacts. It has been shown that an increase in tephra depth on soil may result in the photosynthesis decrease due to lowering of soil oxygen levels, which are important for normal root functioning ([40]). The effect is stronger, when tephra deposit is wet. As a result, the growth of plants may be diminished and the mortality of fine roots may be increased ([40]). The positive effect of leaching of key nutrients (e. g., sulphur, calcium, potassium, magnesium, sodium) is more evident when tephra is deposited in nutrient-poor environments ([21]). However, the functionality of roots and their ability to take up nutrients can be affected, if concentration of Al in the soil increases due to its leaching from tephra ([88]).

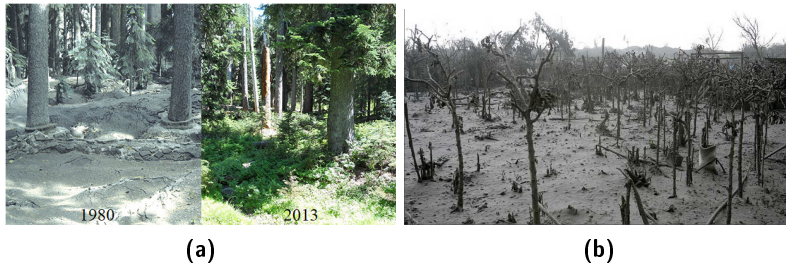


Figure 2.11: (a) - The same forest site in 1980 after the Mount St. Helens eruption, USA, and in 2013. The complete burial of understory by tephra (i. e. ash particles) and survived trees after the ash deposition Joe Antos and Dylan Fischer, www.cbsnews.com; (b) - Broken trees due to tephra (i. e. ash particles) deposition in the Tungurahua province after the Tungurahua eruption in August, 2016 El Comercio www.elcomercio.com.



Figure 2.12: (a) - The tree leaves in the western sector of Tungurahua erupted on 5 November, 2015. V. Lema (IG-EPN) www.igepn.edu.ec; (b) - Crops affected by the tephra (i. e. ash particles) fall from the eruption of Tungurahua on November 14th, 2015 X. Parra (IG-EPN) www.igepn.edu.ec.

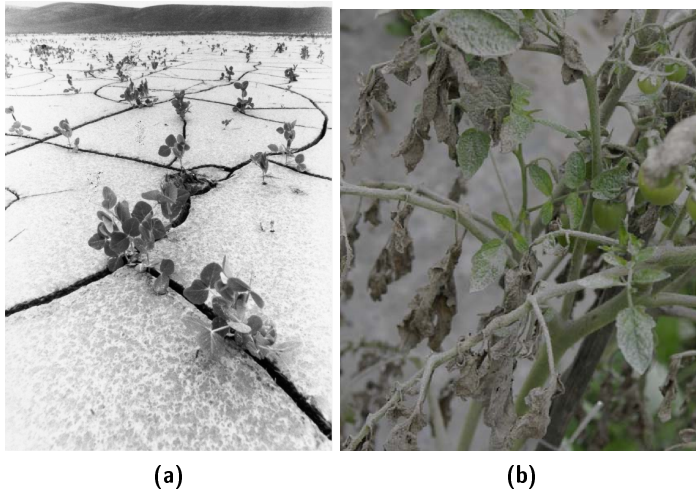


Figure 2.13: (a) - Vegetation reappearance through the cracks of cemented tephra (i.e. ash particles) from the eruption of Mt. St. Helens in 1980 Barry Kough of the Lewiston Tribune www.inland360.com; (b) - Tomato plants presumably damaged due to acid release from tephra (i.e. ash particles) after the eruption of Mt. Merapi, Indonesia in June, 2006 ([86]).

2.4.4 *Physiological effects on vegetation*

Tephra deposition on foliage may affect leaf temperature ([65]; [40]). Most of the damage of the needles of fir trees growing near Mt. St. Helens has been associated with an increase in needle temperatures of about 10.6°C compared to uncovered needles. Although it is expected that the light-coloured tephra typically corresponding to more silicic compositions (Subsection 2.3.2) would reflect visible light, leading to a leaf temperature decrease, [65] and [40] observed the opposite. A reported increase in needle temperature was attributed to the altered geometry of the affected needles (higher leaf surface area, higher leaf boundary layer) that led to the decrease in energy exchanges and, thus,

energy concentration within the needles. The photosynthetic surface of the leaves has been altered by these too high temperatures and growth retardations have been noted after the 1980 eruption, which are related to a loss of photosynthetic potential ([65]).

Tephra can directly alter not only energy exchanges by creating a light barrier (less radiation to the leaves), but also gas exchanges of the leaves. The lower gas exchange can potentially result in a decrease or even halt of photosynthesis, for example, by clogging the leaf stomata, accompanied by an increased leaf abscission ([16]; [40]).

When a volcanic eruption coincides with the flowering period, the buds or flowers may be buried under a layer of tephra, preventing their development and thus causing their damage while still attached to the plant. Some plants may reproduce a new flower cluster at the end of a stem of a shoot, - panicle, as a strategy to divert the damaging effect of tephra. Tephra may also prevent the fruit development and even if some capsules are formed, most plants will bear aborted fruits ([56]; [84]; [85]).

Since the mechanism of photosynthesis involves various components, including photosynthetic pigments (chlorophyll and carotenoid) and photosystems, the electron transport system, and CO₂ reduction pathways, any damage of any of these components caused by a stress (i.e. salinity, drought and high temperature) may reduce overall photosynthetic capacity of a plant, i.e. capacity of converting light energy into a usable chemical energy ([7]).

This chemical energy is stored in carbohydrate molecules, such as sugars, which are synthesized from carbon dioxide and water. Sugars are the end product of photosynthesis used to mechanically support and shape plant cells (cellulose, pectine), store reduced carbon (starch, sucrose), regulate enzyme activity and osmotic pressure (low molecular weight sugars) and provide non-enzymatic antioxidative defense

(flavonoids). Storage and utilization of sugars depend on certain factors such as light, temperature and daylength ([69]). Therefore, if the environmental conditions are not optimal, dysregulation of the photosynthesis may occur, the formation of carbohydrates may be impaired, potentially resulting in the plant stunting ([7]).

Chlorophyll and carotenoids are the principal pigments capable of trapping light energy in photosynthetic organisms. Carotenoids are also known to be able to lose excitation energy relatively rapidly in the form of heat preventing photo-oxidative damage of the plant ([30]). Chlorophyll synthesis may be impaired if the enzymes necessary for chlorophyll production are not operational, if the expression of the genes coding for these enzymes is impaired, if the ultrastructure of the chloroplasts is modified or if there is no sufficient light captured by the leaf surface ([81]). Chlorophyll degradation can occur due to the presence of enzymes, such as chlorophyllase and pheophytinase, occurring during leaf senescence. Senescence is a process that is genetically triggered with leaf age but can also be accelerated due to environmental stresses such as drought, salt stress, heat stress, and others. Senescence consists of the decline of cellular processes, such as photosynthesis, and the hydrolysis of macromolecules, such as proteins and lipids ([48]).

The formation of reactive oxygen species (ROS) compounds is one of the first responses to the onset of senescence or the presence of abiotic stress. Overproduction and accumulation of ROS, such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($HO^{\cdot}$) results in oxidative stress, - a complex chemical and physiological phenomenon that accompanies virtually all biotic and abiotic stresses in higher plants. The term ROS applies for the substances with one or more activated atoms of oxygen, but not always radicals (H_2O_2 is not a radical). Free radicals are any chemical species that exist independently and contain unpaired electron(s). Both ROS and free radicals promote oxidative stress through oxidation of cell compounds. Oxidative stress is there-

fore associated with severe and long-term redox (reduction/oxidation) imbalance due to the lack of electrons. Secondly, it is one of 'stress factors' (similar to salinity, drought and others) damaging cells and triggering signalling and defense reactions. These definitions are related and can be combined ([29]).

Lipid oxidation (lipid peroxidation) is commonly considered as an "hallmark" of oxidative stress. Severe lipid peroxidation leads to the damage of membranes, collapse of their barrier function, followed by disintegration of organelles, oxidation and disfunction of proteins and nucleic acids. Terminal product of lipid peroxidation is malondialdehyde (MDA), very 'aggressive' substance, which is an indicator of oxidative stress ([29]).

Protein oxidation is another "hallmark" of oxidative stress. The modification of proteins leads to the loss of the functions mediated by this organic component, such as specific metabolic, structural, transport or regulatory activities. The most commonly occurring oxidative protein modification is formation of carbonyl groups, i.e. carbonylation ([29]).

Plants have the ability to combat oxidative stress with enzymatic and non-enzymatic antioxidants that scavenge ROS. Main hydrophilic and lipophilic antioxidants in higher plants include glutathione, ascorbate, tocopherol, proline, betaine ([66]).

Overall, the results of previous studies highlight various positive and/or negative effects of tephra deposits on the properties and functioning of natural and agricultural ecosystems. However, our understanding of the mechanisms of the interaction of tephra with different environments remains limited. An experimental approach investigating tephra impacts, in particular effects on soils, plants and microorganisms, their mechanisms and controlling factors, may provide new knowledge on the interactions of tephra in the environment. In turn, this approach may contribute to inform better volcanic crisis manage-

ment decisions.

2.5 THESIS OUTLINE

The PhD research comprises field and laboratory investigations on the interaction of tephra with natural and agricultural environments.

Here, I address three research questions:

1. What are the mechanisms involved in the formation of a crust at the surface of an ash deposit on soil?
2. Does young tephra serve as an ecological niche for fungi and if so, how does the colonization operate?
3. Does an ash deposit on crop plants affect growth and leaf physiology?

The overall structure of the thesis is divided into three parts and takes the form of seven chapters, including this introductory Chapter 1.

Part i gives an overview of the research topic to the reader. Chapter 2 serves to present a background of the research. It discusses the major features of explosive volcanism and describes the physicochemical properties of tephra. An overview of the knowledge on the environmental effects caused by tephra is also provided.

Part ii discusses the results of the laboratory and field investigations. In Chapter 3 I report the findings of an experimental study aimed at unravelling the mechanisms involved in the surface crust of an ash deposit subjected to simulated rainfall. Chapter 4 is a field study conducted at Etna volcano, Italy, during which I monitored the colonization of a recently-emplaced tephra deposit by fungi over a period of 2

years. The impact of an ash layer on crop plant is examined in Chapter 5 and Chapter 6. Two experiments were carried out under controlled conditions in a phytotron. Tomato (Chapter 5) and rice (Chapter 6) plants were exposed to ash and a variety of physiological and biochemical parameters were determined during two weeks.

Part iii serves to conclude the thesis. Chapter 7 summarizes the contributions of this multidisciplinary study to our understanding of the response of vegetation, soil and microorganisms to tephra deposition.

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Part II

DATA CHAPTERS

SURFACE CRUSTING OF A VOLCANIC ASH DEPOSIT UNDER SIMULATED RAINFALL

3.1 ABSTRACT

Surface crusting of volcanic ash fallout following rainfall favours runoff, erosion and lahar initiation. It may also hamper seed germination and depress plant growth. However, ash crust formation is poorly understood. Reconstructed ash deposits from three ash materials (Mt. Merapi, MER, Eyjafjallajökull, EYJA, and San Cristobal, SC) with different particle size distributions and soluble salt contents were subjected to simulated rainfall to investigate the micro-scale morphological modifications of the ash deposit surface exposed to raindrop impact. Micro-craters and micro-peaks formed on the surface of the ash deposits after rainfall initiation. This was accompanied by micromass accumulation in the forms of one or several layers a few tens to hundreds of micrometres thick. Such morphological changes point to structural crust formation. The crusts consisted of a thin layer of tightly packed clay and silt-size ash particles (SC), overlain by loose coarser materials in micro-craters (MER) or by an almost continuous coarse-grained layer (EYJA). In all cases, the surface crust had a reduced porosity compared to that of the bulk material. Depending on ash particle size, crusting was governed by splash, compaction and vertical sorting (MER), vertical particle sorting (EYJA) or compaction (SC). None of the samples presented evidence of particle cementation through secondary salt precipitation. Our results shed a new light on the mechanisms responsible for post-deposition crusting of a natural ash deposit.

3.2 INTRODUCTION

The deposition of volcanic ash produced by explosive eruptions induce various effects on terrestrial environments ([5]). An ash layer on soil may modify surface albedo and thereby, soil temperature and water balance ([12]; [9]; [21]). An ash deposit that is less porous and conductive than the underlying bulk soil also leads to transport limitations. Thus, ash on soil can reduce water infiltration and storage, thereby increasing the risk of run-off and lahar initiation (e.g., [33]; [19]; [34]). Importantly, this effect is enhanced by surface sealing/crusting of the ash deposit ([22]). Soil scientists define a crust as a thin layer of consolidated material at the immediate soil surface with significantly different structural and mechanical characteristics than the underlying bulk soil. A soil crust (or seal when wet) develops in response to temporal and spatial interactions between physical, biological and chemical properties and processes (e.g., [32]; [4]; [20]).

Post-deposition hardening of fine-textured ash deposits to produce a surface crust after wetting has long been reported; it has been held responsible for the impairment of emerging crop seedlings and diminished plant growth (e.g., [36]; [40]; [1], [3]). [26] and [41] pointed to a relationship between surface sealing/crusting of fine-grained ash and lahar frequencies at Ruapehu, New Zealand and Unzen, Japan, volcanoes, respectively. While there is compelling evidence that the surface of a newly-emplaced ash deposit is susceptible to crusting, the formation mechanisms are poorly understood. At Parícutin volcano, Mexico, [36] posited that surface crusting of ash deposits emplaced by the 1943 eruption was largely the result of compaction and sorting of the ash particles by raindrop impact and erosion. In contrast, [40] argued that the continued formation of a surface crust in ash deposited during the 1963 – 65 eruption of Irazú volcano, Costa Rica, was primarily due to evaporation-driven precipitation of halide salts (originally dissolved from the ash surfaces) within the deposit porosity.

There is a plethora of experimental and theoretical investigations that examine the processes and factors governing soil crust development (e.g., [4]; [2], [32]; [39]; [10]). However, no equivalent effort has been made to describe surface crusting of volcanic ash deposits. Here, we report the results of a laboratory experiment aimed at shedding light on the mechanisms involved in ash crust formation. Three laboratory-reconstructed ash deposits of contrasting texture and soluble salt content were subjected to simulated rainfall of constant intensity and kinetic energy but different durations. We document the micro-scale morphological modifications of the surface of the ash deposits and we infer the main mechanisms responsible for crust formation.

3.3 MATERIALS AND METHODS

3.3.1 *Experimental configuration*

Ash material

Three ash samples (Table B.1) collected fresh (i.e. prior to exposure to rain or snow) from different volcanoes were tested. These materials correspond to the 2010 eruptions of Mt. Merapi, Indonesia (MER) and Eyjafjallajökull volcano, Iceland (EYJA), and the 2000 eruption of San Cristobal, Nicaragua (SC). The particle size distributions of the three ash samples as ascertained by laser particle size analysis are shown in Table B.2 and Figure B.1 in the Supplementary material. EYJA is the coarsest ash ($D_{50} = 76.4 \mu\text{m}$; mean $182.43 \mu\text{m}$), whereas MER is the finest ($D_{50} = 29.7 \mu\text{m}$; mean $51.15 \mu\text{m}$). SC is coarser than MER ($D_{50} = 38.9 \mu\text{m}$; mean $76.31 \mu\text{m}$). The three ash samples also differed in their leachate compositions (Table B.3). The concentrations of dissolved anions and cations are the highest in SC; its total dissolved solids (TDS) is ~ 13 and ~ 32 times that of MER and EYJA, respectively. Together, calcium and sulphate account for $\sim 93 \%$ of SC's TDS.

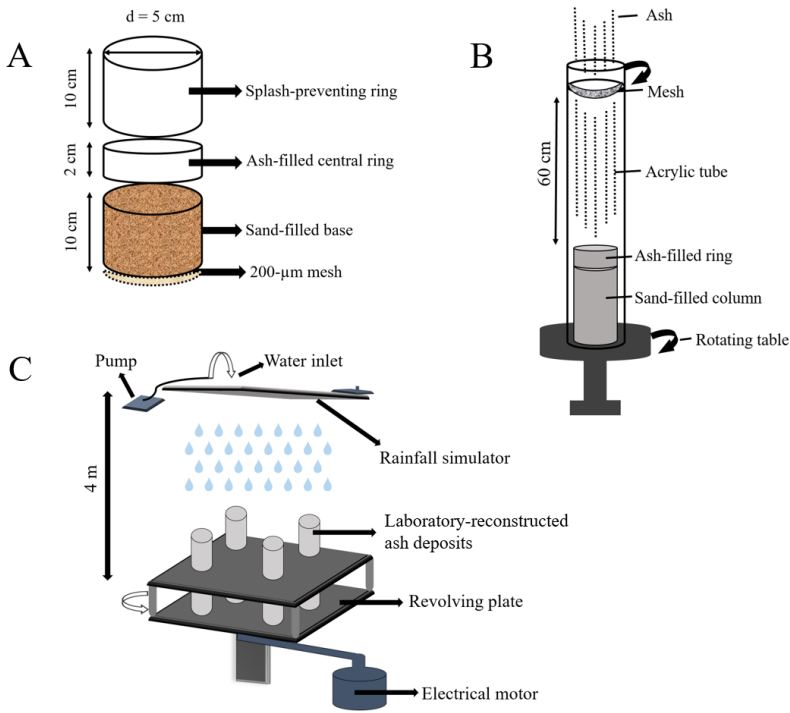


Figure 3.1: Schematics of the 5-cm diameter polyvinyl chloride (PVC) column used to hold the ash deposit (A); the set up used for reconstructing the ash deposit (B); the rainfall simulator placed 4 m above the ash-containing column (C).

Laboratory-reconstructed ash deposit

An ash deposit was reconstructed in the laboratory by modifying a protocol developed earlier for studying soil crusting ([8]). In order to be able to assess changes in the ash deposit following exposure to simulated rainfall, it was important to produce a homogeneous deposit and thus, to avoid particle sorting during its reconstruction. This condition was fulfilled by gently pouring ash through a mesh (1-mm opening) held 60 cm above a two-part Polyvinyl chloride (PVC) column placed

on a rotating table (Figs. 3.1A, 3.1B). The top part of the column consisted of a 2 cm-high ring inside which the ash deposit formed. Previous works on soils, exhibiting various particle sizes, indicate that formation of a surface structural crust is typically restricted to the upper few millimetres ([6]; [7]; [32]; [39]; [10]). Therefore, a ~ 2-cm-thick deposit was considered adequate for identifying crust formation on the ash surface. The bulk densities of the laboratory-constructed MER, SC and EYJA ash deposits were 1.14 ± 0.02 , 1.14 ± 0.01 and 1.25 ± 0.01 g cm⁻³ (mean $\pm$ SE of 24 replicates), respectively. These values are within the range of bulk densities reported for natural ash deposits, e. g., 1.02 – 1.60 g cm⁻³ ([24]; [37]). The ash deposit-containing ring sat on top of a 10-cm high sand-filled base. The sand allowed water to drain and air to escape freely (through a 200- μ m mesh glued to the ring bottom), while preventing water saturation and ponding in the ash deposit itself. Water saturation would affect the transfer of energy from impacting raindrops to the soil surface (e. g., [23]). Finally, losses of ash particles from the deposit surface due to splash erosion upon raindrop impact were prevented by placing another PVC extension on top of the ash-containing ring.

Rainfall simulator

The laboratory-reconstructed ash deposits were exposed to various amounts of rainfall using a simulator modified from [8]. The rain simulator (Fig. 3.1C) consisted of a closed box made of plexiglass with an area of 441 cm². One hundred and forty seven hypodermic needles (diameter 0.45 mm x length 13 mm needle PrecisionGlide, Becton Dickinson and Company), uniformly arranged on the bottom part of the box and spaced 1.5 cm apart, delivered rain drops with a diameter of 2.27 mm from a height of 4 m (i. e. maximum height achievable in the laboratory). The drop velocity was estimated to be 6.17 m s⁻¹ ([14]). To ensure a uniform distribution of raindrop impacts on the ash deposit surface and to avoid drilling, the samples were centered under

the simulator and placed on a slowly revolving plate.

Distilled water was fed to the rain simulator via a peristaltic pump set to produce rainfall with a near-constant intensity of 9.0 ± 0.3 , 10.3 ± 0.8 and 9.4 ± 0.4 mm h⁻¹ for the MER, SC and EYJA ash deposits, respectively. The maximum amount of rainfall (i. e. 20 mm) that could be applied to the deposit without producing water ponding was selected by trial and error. The temporal evolution of surface crusting was assessed by exposing the ash deposits to 5, 10, 15 and 20 mm of water. Since rainfall intensity may vary slightly from one simulation to another, an empty PVC column was used to monitor the actual rainfall intensity. After 20 minutes, rain intensity was determined and if needed, the duration of the simulation was adjusted in order to match the pre-defined rainfall amount as closely as possible. Another empty column placed on the rotating table was used to record the total amount of rain applied to the samples.

In order to verify that changes in the characteristics of the ash deposit exposed to rain resulted from raindrop impact-related processes, a set of samples was covered with a pre-wetted, highly permeable and low water retention capacity Scotch Brite sponge during rainfall simulation. The sponge absorbs the kinetic energy of the falling drops but does not hamper water infiltration. Each combination of ash type ($n = 3$), rainfall amount ($n = 4$) and wetting method ($n = 2$) was replicated three times.

3.3.2 *Post-experiment analyses*

Sample preparation

Following rainfall application, the ash deposit samples were left overnight to allow water to drain. They were subsequently dried at 55 °C for 72 h before impregnation with an epoxy resin (EBL 1466©) under vacuum and at room temperature. The resin-embedded samples were

cut vertically. One-half was impregnated a second time with a high quality, transparent resin (Epoxicure 2©). Thin sections were prepared from slabs cut from the consolidated material and analysed by optical microscopy. The other half of the resin-embedded samples was used to make polished sections.

The polished sections were examined by optical microscopy at magnifications of 0.7x and 1.6x prior to selecting representative sample areas for Scanning Electron Microscopy (SEM) analysis. The corresponding polished sections were cleaned in an ultrasonic bath and coated with gold prior to analysis using a Tescan-Vega LMU SEM instrument. Backscattered electron micrographs were obtained at 25 kV.

Micro-morphology analysis

The extent of the vertical reorganisation of particles close to the ash deposit surface was assessed as follows. The maximum and minimum *Thickness of fine-grained surface layer* (T_{fgl}) and *Thickness of the coarse-grained surface layer* (T_{cgl}) of the samples exposed to the raindrop impact were estimated under the optical microscope on each of three thin sections and the average value of these results was taken. The total thickness of these layers was calculated by summing up the average thickness values of the fine- and coarse-grained layers.

A cross-sectional image of each ash deposit was obtained by stitching the SEM images together¹. Depending on magnification settings on the SEM, the pixel size was 0.48 μm or 1.28 μm (MER and SC), and 0.7 μm or 1.89 μm (EYJA). Colour segmentation of the composite SEM images was performed using a k-means clustering algorithm². The resulting 12-level colour images were then thresholded manually to produce three-level greyscale images. Pore space and ash particles were assigned to the black (B) and white pixel classes (W), respectively,

¹ Fiji© software, <https://fiji.sc>

² QGIS©, www.qgis.org; [18]

whereas objects that could not be defined with certainty were assigned to the grey pixel class (G). The minimum percentage pore area was calculated according to:

$$\theta_{\min} = \frac{B}{B + W + G}.$$

Assuming that the proportion of pore space in the grey class is the same as that defined by the ratio ($\frac{B}{B+W}$), then the percentage pore area in the grey class (θ_G) is given by:

$$\theta_G = \frac{G \times B}{(B + W + G) \times (B + W)}.$$

The θ_G value was added to $\theta_{\min}$ to infer the maximum percentage pore area ($\theta_{\max}$). A porosity-depth profile of the ash deposit was generated using a Python³ script (Listing B.1). The computed porosities were smoothed using a 15-point moving average. The difference between the lower and upper porosity ranges between 1 and 8%. We performed the porosity calculations on the stitched SEM image obtained for each experimental sample. Finally, a rough estimate of ash particle size was obtained from the longest particle dimension as inferred from the SEM images. Forty measurements were performed to describe particle size in any area of interest, and three SEM images for each experimental sample were processed. Ash particles that were too small for size estimation by this method were assumed to represent clay-size particles.

Micro-topography analysis

The micro-topography of the ash deposit surface subjected to rainfall was described in terms of spacing and amplitude of surface features (micro-peaks and micro-craters) (Fig. 3.2). *Micro-peak counts* (P_c) and *Micro-crater counts* (C_c) in # peaks or # craters cm^{-1} , respectively, were

³ www.python.org

determined from photos of the deposit surfaces (Fig. 3.3). The evaluation length used was typically 4 cm, i.e. the edges of the deposit surface were excluded. The *Maximum crater to peak height* (R_{cp}) is the vertical distance between the top of a micro-peak and the bottom of a micro-crater (Fig. 3.2). An average R_{cp} value was obtained for three replicate samples from the analysis of a portion (~ 4 cm in length) of the thin sections.

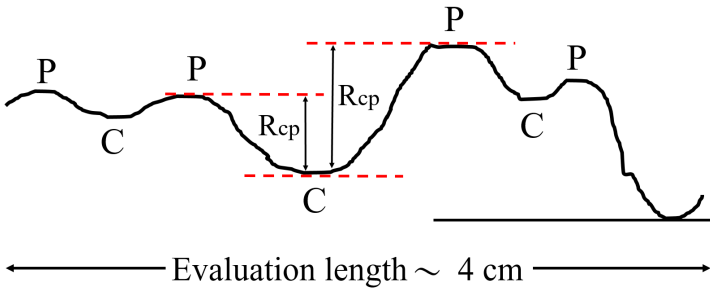


Figure 3.2: Schematic representation of the micro-topography of the ash deposit surface subjected to raindrops: micro-peak (P_c), micro-crater (C_c) and vertical distance between top of a micro-peak and bottom of a micro-crater (R_{cp}).

Statistics

The porosity values of bulk and surface layer were compared by means of a paired t-test, with a p value of 0.05. A one-way analysis of variance (ANOVA1) in combination with a Tukey's test ($p < 0.05$) was performed to test C_c , P_c and R_{cp} for significant differences across rainfall amounts (5, 10, 15 and 20 mm).

3.4 RESULTS

Visually, only the ash deposits subjected to direct raindrop impact showed signs of particle rearrangement at the vicinity of the surface.

Below, we focus specifically on this set of samples. The results of the surface micro-topography and morphological measurements are compiled in Table B.4.

3.4.1 *Micro-topography*

MER ash deposit

The surface of **MER** ash deposit changed immediately after the onset of rainfall (Fig. 3.3A). Ash pellets formed rapidly, but these structures were transitory as they disappeared when the surface became moist and riddled with micro-peaks and micro-craters. After 5 mm of rainfall, P_c and C_c were on average 2.1 ± 0.2 and $2.0 \pm 0.2 \text{ cm}^{-1}$, respectively. These values decreased by more than 40 % at higher cumulative rainfall, coinciding with smoothening of the deposit surface. At this point, the ash deposit surface became “shinny”, suggesting gradual water saturation. After 20 mm of rainfall, P_c and C_c were less than 1 cm^{-1} . R_{cp} increased from $1.3 \pm 0.1 \text{ mm}$ (5 mm of rainfall) to $1.9 \pm 0.1 \text{ mm}$ (10 mm of rainfall), and then decreased to $1.6 \pm 0.2 \text{ mm}$ after 20 mm of rainfall. Water ponding did not occur.

SC ash deposit

Similar to **MER**, transient ash pellet formation occurred at the surface of **SC** deposit as soon as rain started (Fig. 3.3B). Five millimetres of rainfall resulted in riddling of the surface with micro-peaks and micro-craters ($P_c = 2.0 \pm 0.1 \text{ cm}^{-1}$, $C_c = 1.7 \pm 0.2 \text{ cm}^{-1}$). P_c decreased significantly after 15 mm of rainfall and was close to 1 cm^{-1} at the end of the experiment. C_c did not vary after 10 and 15 mm ($1.6 \pm 0.1 \text{ cm}^{-1}$; $1.4 \pm 0.1 \text{ cm}^{-1}$, respectively) of rainfall, but it decreased slightly after 20 mm of rainfall ($1.0 \pm 0.2 \text{ cm}^{-1}$). Water infiltration through the deposit became more sluggish after 15 mm of rainfall, although ponding did not occur. In contrast to **MER**, R_{cp} significantly increased from $1.5 \pm 0.1 \text{ mm}$ (5 mm of rainfall) to $2.1 \pm 0.2 \text{ mm}$ (20 mm of rainfall).

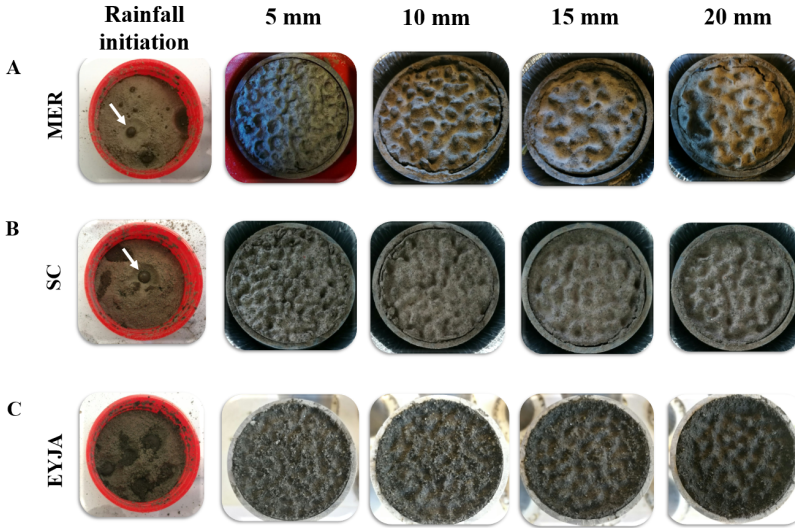


Figure 3.3: Photographs of the surface of **MER** (A), **SC** (B) and **EYJA** (C) ash deposits after exposure to 5, 10, 15 and 20 mm of rainfall. The white arrows point to ash pellets formed at the **MER** and **SC** deposits surface immediately after rainfall initiation.

EYJA ash deposit

Pellet formation was minimum for **EYJA**, and micro-peaks and micro-craters did not form on the deposit surface after 5 mm of rainfall (Fig. 3.3C). With additional rainfall, micro-peaks and micro-craters appeared. However, P_c , C_c and R_{cp} did not change significantly between 10 and 20 mm of rainfall. There were no visible signs of decreased water infiltration during the experiment.

3.4.2 *Micro-morphology*

Cross-sectional **SEM** images of the rain-protected **MER**, **SC** and **EYJA** samples did not show any signs of particle reorganisation at the vicinity

of the deposit surface (Fig. B.2). This contrasted with the ash deposits directly exposed to raindrop impacts.

MER ash deposit

The thin sections and SEM images clearly highlighted particle reorganisation at the vicinity of the surface of MER deposit. After 5 mm of rainfall this manifested as a thin ($\sim 70\text{--}80\text{ }\mu\text{m}$) layer comprised of closely packed fine ash particles extending laterally across the deposit surface, although mostly in micro-craters (Fig. 3.4 - 3.4A). Occasionally, coarser particles (thickness $\sim 120\text{ }\mu\text{m}$) were observed at the surface in micro-craters. The SEM images indicated that the thin layer consisted predominantly of silt- and clay-size particles (Fig. 3.5A). It further developed after 10 mm of rainfall (Fig. 3.4B). In micro-craters, coarser material (fine and very fine sand) continued to accumulate on top of the thin layer (Fig. 3.5B). With additional rainfall, multi-layered structures appeared, again more prominently in micro-craters (Figs. 3.4C and 3.5C). It consisted of silt- and clay-size particle micromasses alternating with sand-size materials. The multi-layered structures continued to develop until the end of the rainfall simulation (Figs. 3.4D, 3.4E and 3.5D), by which time they had a total thickness of up to $\sim 1.26\text{ mm}$ (Table B.4). In parallel, more coarse particles accumulated at the surface of the ash deposit. Careful examination of the SEM images did not provide evidence for chemical cementation of ash particles in the deposit surface.

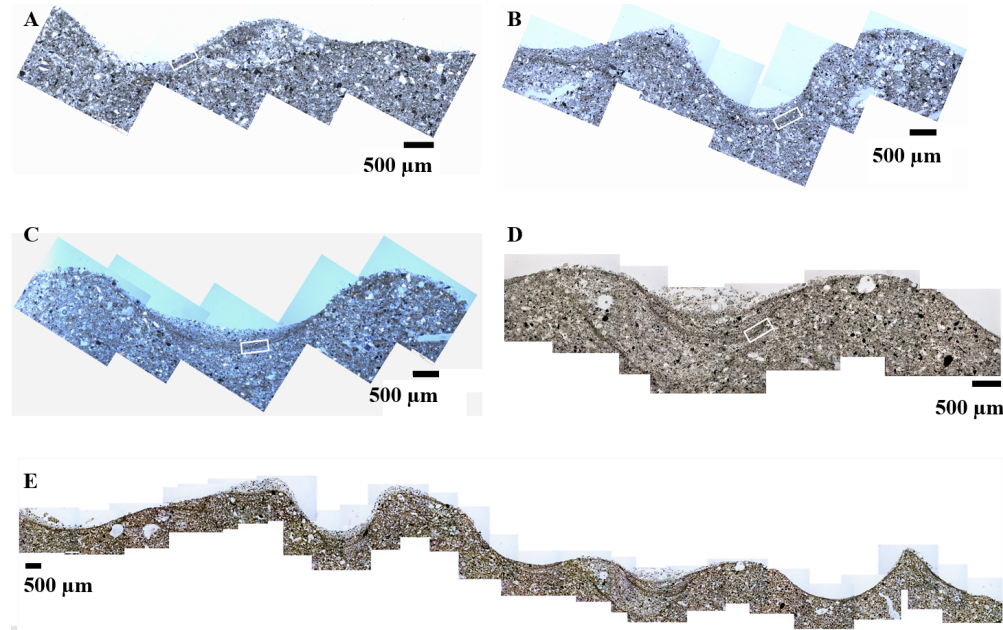


Figure 3.4: Optical microscope images (stitched) of the thin cross sections of the MER ash deposit after exposure to 5 mm (A), 10 mm (B), 15 mm (C) and 20 mm (D) of rainfall. The rectangle outlined in white highlights the location of the thin layer comprised of tightly-packed ash particles. The representativity of the observed particle reorganisation along the sample is demonstrated through the image of ~ 4 cm cross section of the MER deposit exposed to 20 mm of rainfall (E).

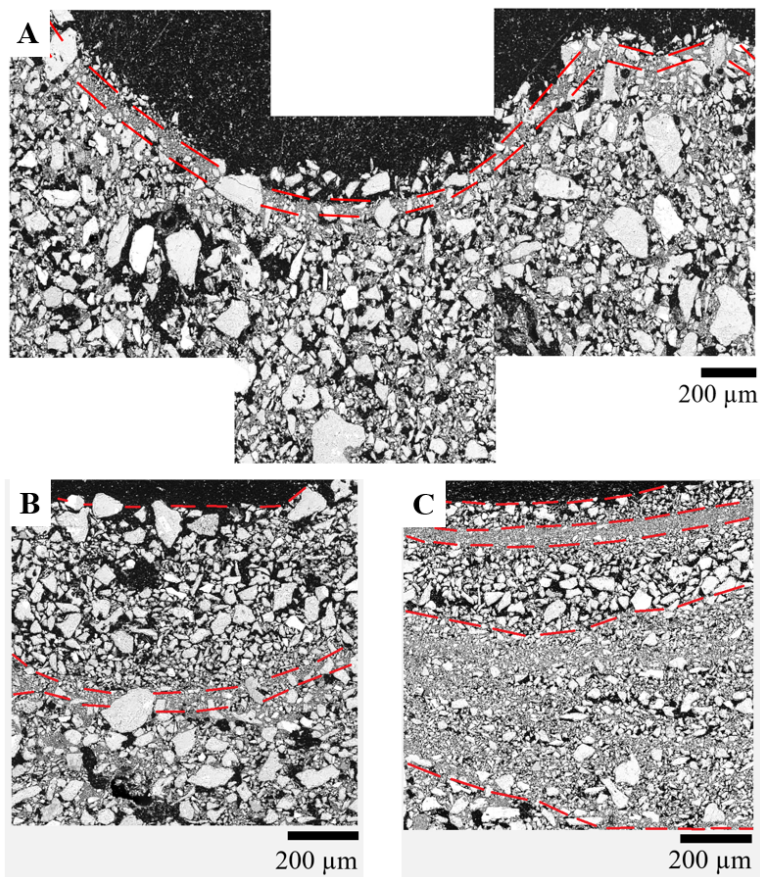


Figure 3.5: SEM images of polished cross sections of the MER ash deposit after exposure to 5 (A), 10 (B) and 20 mm (C) of rainfall. The red dashed lines delineate the near-surface layer affected by particle reorganisation in micro-craters. The ash material located below this layer corresponds to the unaffected bulk deposit.

SC ash deposit

Similar to [MER](#), application of 5 mm of rainfall to [SC](#) ash deposit led to particle reorganisation and appearance of a thin ($\sim 40\text{--}50\text{ }\mu\text{m}$) layer of tightly packed clay-size particles, more prominently in micro-craters (Figs. [3.6A](#) and [3.7A](#)). The thin layer became mostly laterally continuous at higher cumulative rainfall amounts, although some micro-peaks remained unaffected (Figs. [3.6B-E](#)). After 10 and 15 mm of rainfall, the thin layer was dominated by clay- and fine and coarse silt-size particles (Figs. [3.7B-D](#)). Independently of the rainfall amount, coarser particles occurred occasionally in micro-craters and micro-peaks on top of the thin layer. Analysis of the [SEM](#) images indicated that the coarse material was dominated by very fine sand-, fine silt- and coarse silt-size particles. At the end of the rainfall simulation, the total thickness of the deposit affected by particle reorganisation was $120\text{ }\mu\text{m}$ when the layer of coarse particles was absent; it nearly doubled when it was present (Table [B.4](#)). Particle reorganisation did not occur in the bulk material beneath the surface (Fig. [3.7E](#)) and secondary chemical precipitates were not detected in deposit surface.

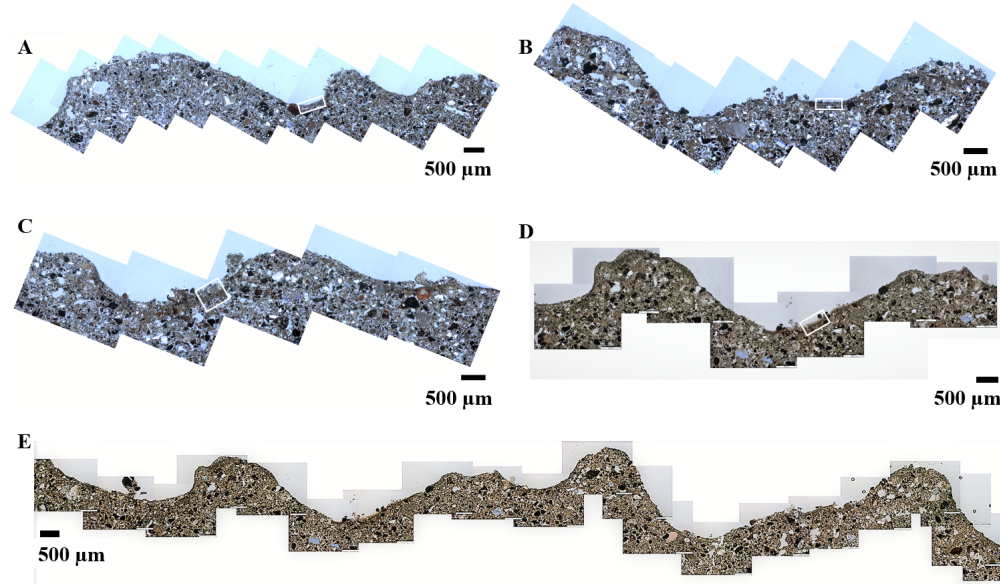


Figure 3.6: Optical microscope images (stitched) of the thin cross sections of the SC ash deposit after exposure to 5 mm (A), 10 mm (B), 15 mm (C) and 20 mm (D) of rainfall. The rectangle outlined in white highlights the location of the thin layer comprised of tightly-packed ash particles. The representativity of the observed particle reorganisation along the sample is demonstrated through the image of ~ 4 cm cross section of the SC deposit exposed to 20 mm of rainfall (E).

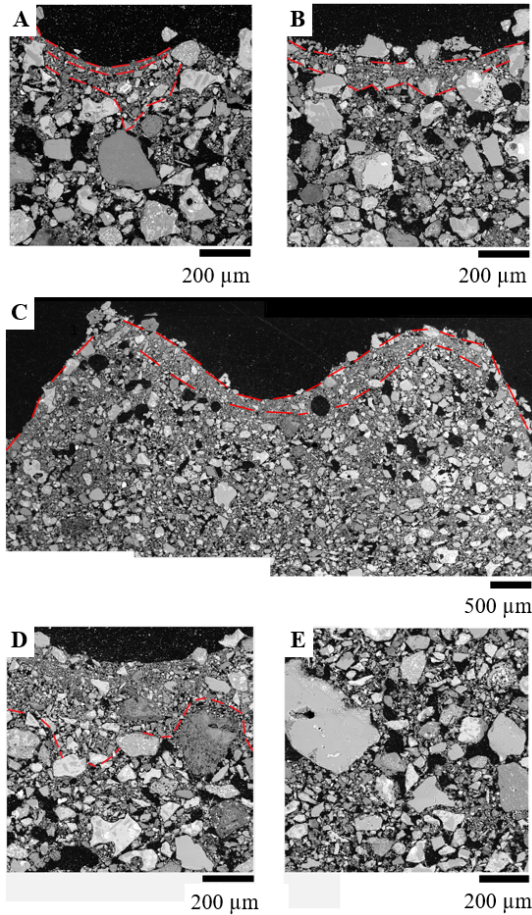


Figure 3.7: SEM images of polished cross sections of the SC ash deposit after exposure to 5 (A), 10 (B) and 15 mm (C and D) of rainfall. The red dashed lines delineate the near-surface layer affected by particle reorganisation in micro-craters (A), (B) and (D), and in micro-craters and on micro-peaks (C). The ash material located below this layer corresponds to the unaffected bulk deposit (E).

EYJA ash deposit

Although more erratically than in [MER](#) and [SC](#), the thin sections and [SEM](#) images also highlighted particle reorganisation at the vicinity of the surface of [EYJA](#) deposit (Figs. [3.8A-E](#) and [3.9A-C](#)) compared to the bulk layer unaffected by drop impact (Fig. [3.9D](#)). Again, cementation of ash particles in the rain-treated deposits did not occur. Analysis of the thin sections and [SEM](#) images of the samples exposed to 20 mm of rainfall revealed loose accumulation of fine to coarse sand-size particles at the surface, forming a $\sim 820 \mu\text{m}$ thick band (Table [B.4](#)). A discontinuous, $\sim 230 \mu\text{m}$ -thick layer made of tightly packed clay-, fine silt-, coarse silt- and very fine sand-size particles, was observed immediately below the coarser material accumulation. At lower cumulative rainfall amounts, the formation of the thin layer of tightly-packed particles was less clear (Figs. [3.8A-C](#)) and was confirmed only in micro-craters (Figs. [3.8A-C](#)). In contrast to [MER](#) and [SC](#), the thickness of the deposit affected by particle reorganisation did not vary with cumulative rainfall (Table [B.4](#)).

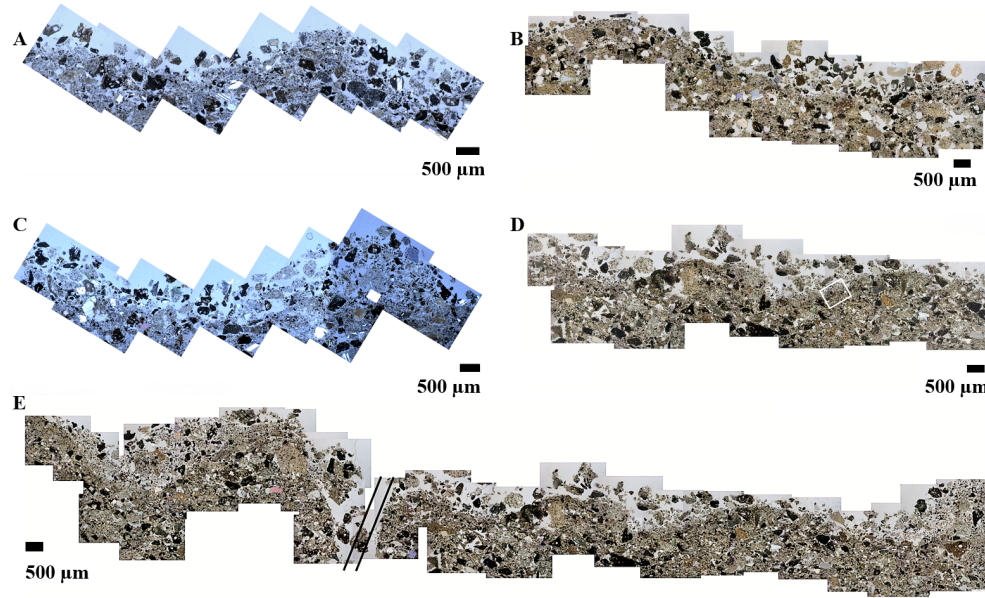


Figure 3.8: Optical microscope images (stitched) of the thin cross sections of the [EYJA](#) ash deposit after exposure to 5 mm (A), 10 mm (B), 15 mm (C) and 20 mm (D) of rainfall. The rectangle outlined in white highlights the location of the thin layer comprised of tightly-packed ash particles. The representativity of the observed particle reorganisation along the sample is demonstrated through the image of ~ 4 cm cross section of the [EYJA](#) deposit exposed to 20 mm of rainfall (E).

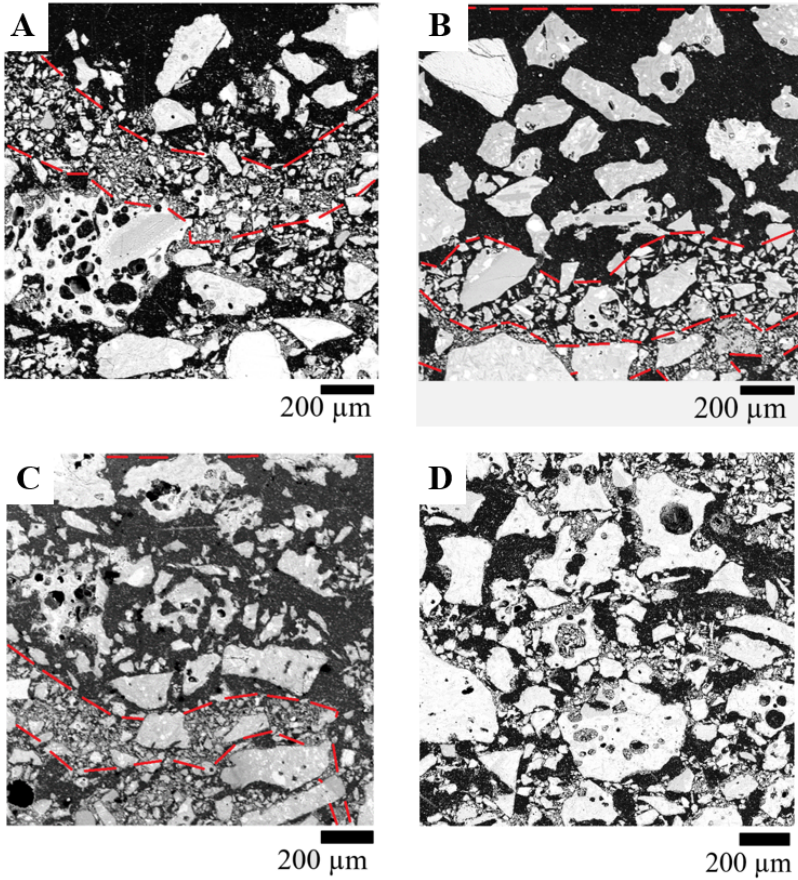


Figure 3.9: SEM images of polished cross sections of the EYJA ash deposit after exposure to 5 (A), 10 (B) and 15 mm (C) of rainfall. The red dashed lines delineate the near-surface layer affected by particle reorganisation in micro-craters (A), (B) and (C). The ash material located below this layer corresponds to the unaffected bulk deposit (D).

3.4.3 Porosity measurements

The percentage pore area measurements in the MER, SC and EYJA samples are reported in Table B.5 and illustrated in Figures B.3 to B.5.

MER ash deposit

The thin layer of closely packed silt- and clay-size ash particles, which formed at the vicinity of the surface of MER deposit upon exposure to rainfall showed a reduced porosity (up to 69 % in relative units) compared to that of the subsurface bulk material. The difference was statistically significant ($p < 0.05$), irrespective of rainfall amount. In contrast, a comparatively higher (~ 30 % in relative units) porosity were inferred for the coarser material found on top of the thin layer.

SC ash deposit

Similar to MER, the porosity of the dense thin layer at the vicinity of the surface of SC sample exposed to 5, 10, 15 and 20 mm of rainfall was lower (by up to 58 % in relative units) than that measured for the bulk part of the deposit material.

EYJA ash deposit

Compared to the bulk deposit material, the porosity of the thin layer was lower by 16-49 % (in relative units) after 10 to 20 mm of rainfall. In contrast, the layer of coarser particles (identified at 10 and 15 mm rainfall) above the thin layer exhibited 18-39 % higher porosity than that of the bulk ash deposit.

3.5 DISCUSSION

3.5.1 *Changes in the surface micro-topography of the ash deposit*

Raindrops impacting the dry surfaces of MER and SC ash deposits caused the transient formation of pellets (Figs. 3.3A and 3.3B). Similarly, [22] reported rapid, but short-lived, appearance of pellets at the surface of an ash bed subjected to simulated rainfall. By analogy with soils subjected to rainfall ([27]), ash pellet formation may relate to particle hydrophobicity. With increasing addition of rainwater, the ash became more hydrophilic, impeding further pellet development. The process stopped as the entire ash deposit surface wetted up. The hydrophobicity of volcanic ash is poorly documented. [25] argued that hydrophobic ash is generated by eruptions with a dominantly magmatic rupture mechanism, whereas hydrophilic ash is preferentially associated with eruptions with a strong phreatomagmatic component (i. e. involving magma-water interactions). MER ash belongs to the former style of eruption, whereas EYJA ash corresponds to the latter (Table B.1, [17]). The case of SC is less clear as this ash contains hydrothermal minerals and may have emitted as a result of phreatic and strombolian explosions ([16]). Interestingly, ash pellets did not occur upon wetting of EYJA deposit (Fig. 3.3C), possibly reinforcing the idea that hydrophobicity plays an important role in their formation. Alternatively, the coarser texture of EYJA ash (Table B.2 and Fig. B.1) may have facilitated water penetration as raindrops hit the deposit surface, thereby preventing pellet development.

Raindrop impacts rapidly led to the formation of micro-craters and micro-peaks at the surface of the ash deposit after 5 mm (MER and SC) and 10 mm (EYJA) of cumulative rainfall. Interestingly, C_c and P_c tended to decrease with cumulative rainfall applied to MER and SC (Table B.4), revealing an increase micro-peaks' and micro-craters' width over time. This may have resulted from a change in the mechanical properties of the ash material, which, upon gradual saturation of the

deposit, increasingly behaved as a rigid layer when falling raindrops impinged the surface ([31]). A more rigid surface tends to decrease the angle formed by the surface and the lateral jets of water generated upon raindrop impact ([13]). Jets that are more horizontal will affect a larger radius around the point of raindrop impact, potentially accounting for the observed micro-crater widening. This sequence of events may have been particularly efficient in the cases of MER and SC, which both rapidly developed a thin layer of closely packed fine ash particles at the vicinity of the surface hit by raindrops and showed signs of water saturation (but not ponding) near the surface during the simulated rainfall.

3.5.2 *Evidence for surface crusting of the ash deposit*

Ash particle cementation by mineral precipitates has been invoked to explain the presence of a crust at the surface of an ash deposit (e.g., [40]). Owing to its extremely high concentrations of dissolved calcium and sulphate (Table B.3), SC ash was the most likely candidate that could have accommodated precipitation of an efficient cementing agent such as calcium sulphate. However, this was not confirmed by SEM analysis observations. This is probably due to rapid leaching of the soluble ions released from ash through the deposit and the underlying sand layer. Based on this result, we exclude salt precipitation as a main crust formation mechanism in our experiments.

So-called structural crusts form at the soil surface in the absence of runoff. Since in our experiments ponding did not occur and the deposit surfaces were kept horizontal, lateral redistribution and deposition of particles by runoff can be discarded as a potential crust formation mechanism. Thus, structural crusts formed on soils probably represent a convenient model for investigating surface crusting of an ash deposit. In our study, exposure of the MER, SC and EYJA ash deposits to direct raindrop impact clearly led to particle reorganisation at

the vicinity of the deposit surface (Figs 3.4-3.9). Particle reorganisation was a dynamic process as it rapidly evolved with cumulative rainfall. The most conspicuous changes were the appearance of micro-craters and micro-peaks shortly after initiation of rainfall, and the accumulation of micromass in the forms of one or several layers a few tens to hundreds of micrometers thick. Such morphological features typically denote development of a structural crust (e.g., [32]). Moreover, the tightly-packed layer of fine particles found close to the surface of MER and SC displayed a systematic and significant reduction in porosity (Table B.5).

Similar to what has been reported for coarse-textured soils or artificial mixtures dominated by sand fractions of various sizes ([39]; [6], [7]), we argue that crust formation at the surface of the ash deposit was strictly dependent upon the energy input from drop impact. Our results, which show that the samples protected from direct raindrop impact did not exhibit any topographical or morphological modifications of their surfaces (Fig. B.2), support this view. Consequently, the forces involved in wetting (i.e. physico-chemical dispersion) and water infiltration (i.e. detachment by water shear stress and subsequent particle transport) alone were insufficient to induce surface crusting.

3.5.3 *Mechanisms of crust formation at the surface of the ash deposit*

Spatial differentiation of a structural crust in soils subjected to raindrop impact is the result of solid matter translocation. While ash aggregates that formed in volcanic plumes can be incorporated in natural ash fallout deposits ([11]), they (if initially present in the original ash materials) were most likely destroyed during the construction of the laboratory ash deposits. Convincingly, ash aggregates were never observed in our samples and we dismiss a potential contribution of comminution of aggregates by wetting (i.e. slacking) to ash crust formation. For the same reasons, aggregate breakdown by raindrop im-

pact is not considered further. We also posit that particle dispersion driven by physico-chemical conditions did not play a significant role as crusting was not observed on samples protected from raindrop impact. This most likely reflects the absence of particles with significant surface electrical charges in the ash deposits.

Four main mechanisms can potentially contribute to particle reorganisation during raindrop impact on non-aggregated material, such as the surface of our laboratory-reconstructed ash deposits. These include:

1. compaction in response to the compressive stress generated during raindrop impact. The particle size distribution of the compacted layer remains identical to that of the original material, but the overall porosity is reduced ([28], [29]; [15]);
2. particle eluviation/illuviation: as water penetrates into the ash deposit during drop impact, small particles may be entrained by hydraulic jets and the infiltrating water, leading to particle eluviation ([10]; [19]). These particles may then accumulate again at some depth below the deposit surface (illuviation);
3. particle sieving, i.e. the downward percolation of small particles in between coarser particles which results from the horizontal shear strain caused by raindrop impact ([38]; [10]; [6], [7]). This mechanism is applicable to granular media with limited cohesion between particles. Contrary to compaction, mechanisms 2 and 3 affect particle size distribution, with a relative enrichment in coarse particles near the surface (washed-out layer) and a relative enrichment in finer particles at some depth below the surface (washed-in layer);
4. splash, i.e. upon raindrop impact, droplets loaded with particles are ejected outwards, leading to lateral redistribution of material ([23]).

Mechanisms 1, 2 and 3 produce a vertical reorganisation of particles, whereas mechanism 4 leads to horizontal particle redistribution.

MER ash deposit

A well-developed crust formed at the vicinity of MER deposit surface (Figs. 3.4 and 3.5). As the experiment progressed, the crusts formed in micro-craters and on micro-peaks became more and more differentiated. From a morphological point of view, the crust associated with micro-peaks evolved little with additional rainfall (Table B.4). In contrast, both the morphology and total thickness of the crust formed in micro-craters changed significantly. Noticeably, a multi-layered crust developed in micro-craters after 15 mm of rainfall (Fig. 3.5C). The generation of differentiated crusts implies that the surface micro-topography remained reasonably stable after some time, despite raindrops hitting the surface randomly. In other words, micro-craters that formed immediately after rainfall initiation acquired a stable position as the deposit surface wetted up and acquired a non-deformable behaviour. The increased mechanical strength of the surface as a result of wetting and/or structural crust formation may explain why the surface micro-topography evolved towards a fairly stable configuration.

On micro-peaks, the compressive stress resulting from raindrop impingement probably led to surface compaction and hence, decreased porosity (Table B.5). In parallel, shear stress caused by hydraulic jets during raindrop impact led to the splashing of particles outwards from the impact center. Thus, raindrop impact can initiate a crust but can also partly disrupt it. While there were no direct signs of vertical particle segregation on micro-peaks, washed-out coarse grains from the micro-peaks may have been trapped in micro-craters after being transported by splash, thereby contributing to the loose accumulation of coarse material on top of the thin layer of silt- and clay-size particles in micro-craters. In soils, vertical particle segregation is typically associated with coarse textures ([6]; [39]). Thus, the absence of a washed-out

layer in micro-peaks may relate to the relatively small content of coarse particles in MER ash (Table B.2). Raindrops affected the whole deposit surface evenly and therefore, the initial stages of crust development in micro-craters and on micro-peaks were probably governed by similar mechanisms. This is supported by a morphological resemblance between the thin layer of silt- and clay-size particles, which formed at the bottom of the micro-craters and on micro-peaks (Fig. 3.5A). For higher rainfall amounts, additional material enriched in coarser particles accumulated in micro-craters possibly due to preferential ejection of fine particles during splash ([30]) and trapping of the splashed (coarser) material. As shown in Fig. 3.5B, vertical particle segregation affected the ash material amassed in micro-craters. The vertical sorting of sand-size particles by raindrop impact is a phenomenon occurring in coarse-textured soils ([38]; [35]). It is likely the consequence of particle sieving and/or eluviation-illuviation. Since the depth affected by raindrop impact is relatively limited ([6]), the repeated accumulation of material and particle segregation led to the smoothening of deposit surface (Table B.4) and to the development of multi-layered crusts at the vicinity of the MER surface deposit, as observed after 15 and 20 mm of rain (Fig. 3.5C).

SC ash deposit

There were no clear signs of vertical particle segregation, neither on the micro-peaks nor in the micro-craters formed at the surface of SC deposit. Hence, crust formation after 5 mm of rainfall was probably driven mainly by compaction and splash. However, in contrast to MER, the thin layer of tightly packed fine particles was discontinuous (Figs. 3.6 and 3.7), possibly revealing a small shift in the balance between crust forming and crust destruction (by splash) mechanisms at the beginning of rainfall simulation. Such shift may relate to the somewhat coarser texture of SC ash (Table B.2), which would allow a deeper, and therefore more disruptive, impact of raindrop on the deposit surface

([6]).

In general, a coarse texture facilitates the downward movement of fine particles. However, vertical particle segregation occurred only occasionally in the SC samples, despite a slightly coarser texture than MER (Table B.2). Interestingly, the latter is characterized by a more marked mode in the fine sand-coarse silt fraction (Fig. B.1). It also exhibits a small secondary mode around 200 μm . In contrast, SC displays a rather homogeneous particle size distribution. The presence of strong modes in the particle size distribution of MER may have facilitated downward particle movement, and hence segregation, whereas the more homogeneous particle size distribution of SC ash may have impeded it.

Finally, larger rainfall amounts did not cause prominent changes in SC crust features. In particular, multiple layering of coarse and fine material seldom took place in micro-craters. This is reflected in the similar crust thickness both in micro-craters and on micro-peaks (Table B.4).

EYJA ash deposit

Crusting of EYJA, the coarsest sample among the studied ash materials (Table B.2), was erratic and less developed than for MER and SC. Washed-out coarse particles were apparent at all stages in micro-craters (Fig. 3.9A-C), and sometimes on micro-peaks, forming a layer up to 1 mm thick after 20 mm of rainfall (Table B.4). Despite the presence of a washed-out layer, micromass accumulation of tightly packed fine particles barely occurred in the EYJA deposit during the early stages of crust development. While this feature developed at higher rainfall amounts, it remained mostly discontinuous (Fig. 3.8). Similarly, [22] noticed that rainfall did not lead to surface crust formation in ash dominated by coarse particles ($D_{50} = 525.8 \mu\text{m}$) although it did in a finer ash sample ($D_{50} = 54.3 \mu\text{m}$).

We postulate that the coarse ash texture, and resulting larger pore sizes and lower water content at all stages of the rainfall simulation, probably allowed water to penetrate easily into the deposit during raindrop impact. Thus, less energy was available for compaction and this mechanism probably did not contribute significantly to crust formation.

The coarse texture, as well as the presence of a strong mode around 350 μm in the particle size distribution (Fig. B.1), may have favoured particle sieving and eluviation-illuviation as the main mechanisms leading to crust development in EYJA ash deposit. The comparatively slow rate of formation and discontinuous nature of the dense thin layer at the vicinity of the EYJA deposit is surprising. This could be an effect of ash particle shape; the ash material tended to be angular in EYJA whereas more round shapes characterised MER and SC samples (e.g., Figs. 3.5, 3.7 and 3.9). Ash angularity may interfere with and impede the downward movement of small particles. It could also explain a lesser sensitivity to compaction.

3.6 CONCLUDING REMARKS

Exposure of three reconstructed ash deposits to simulated rainfall led to the formation of a structural crust at the vicinity of the deposit surface. The crust was typically comprised of a thin layer of tightly packed clay and silt-size ash particles overlain by loose coarser materials. The thin layer exhibited a lower porosity compared to bulk soil. We infer that crusting was governed primarily by ash particle reorganisation in response to raindrop impact rather than by particle cementing through secondary salt precipitation. In MER, rain splash-driven particle detachment and compaction by raindrop impact initiated crust formation at the onset of rainfall. Sieving and eluviation/illuviation mechanisms became more important with additional rainfall, leading to vertical particle sorting and development of a multi-layered and

thicker crust. In contrast to [MER](#), particle sieving and eluviation/illuviation were subdued in [SC](#) ash, and crust formation through compaction dominated. We interpret these behaviours as a consequence of different particle size distributions in [MER](#) and [SC](#), the former material being slightly richer in clay and silt fractions. In the case of [EYJA](#), its coarse texture probably favoured rainwater percolation through the deposit and hence, particle sieving and eluviation/illuviation. This produced a discontinuous crust in the deposit surface.

Contrary to previous assumptions, our study does not support salt precipitation as a main crust formation mechanism. In any case, cementing of the ash surface due to salts is unlikely when the conditions (rainfall amount, ash hydraulic conductivity) allow for rapid leaching of soluble salts. Instead, we emphasize the important role of particle size distribution of an ashfall deposit in dictating susceptibility to crusting. Ash fall deposits are usually size-sorted and the finer material is typically transported and deposited farther away from the volcano. We show that a fine-grained material is more prone to surface crust formation upon raindrop impact and therefore, we may anticipate a differential impact with distance from the volcano; i.e. a distal, thin ash deposit could be more detrimental to the soil-atmosphere exchanges than a proximal, thicker ash deposit. Moreover, since aggregate dispersion during raindrop impact is recognised as an important component of physical crust formation in soil (e.g., [\[32\]](#); [\[39\]](#); [\[2\]](#)), the presence of ash aggregates in a deposit will likely promote surface crusting.

Overall, our study provides novel insights for interpreting post-deposition crusting of a natural ash deposit. We present benchmark experimental data on ash crust formation and concomitant reduction in porosity, which can also aid in the understanding of the factors controlling the initiation of rainfall-triggered lahars ([\[22\]](#)). Future experiments similar to those designed and developed here could encompass a broader range of ash types and rainfall parameters (for example,

wetting-drying cycles). This would further deepen our knowledge of the residence time of volcanic ash on soil.

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FRESH TEPHRA DEPOSITS PROVIDE A HABITAT FOR RAPID FUNGI COLONIZATION: A CASE STUDY FROM MT. ETNA

4.1 INTRODUCTION

The episodic depositions of volcanic material -tephra- on terrestrial ecosystems drastically affect soil microbial activity as it has direct impact on soil properties and environmental conditions such as temperature, water drainage or moisture content. The extent of these effects depends primarily on the tephra colour (albedo change), porosity, pore size distribution, thickness but also on its chemical composition and residence time on soil ([7]). In contrast to soil, the freshly-deposited tephra is almost devoid of organic matter and organic nitrogen, which limit microbial colonization and activity ([50]). Nonetheless, young volcanic deposits represent a substrate that microorganisms readily colonize ([36]; [35]; [42]; [41]; [50]) despite being exposed to high solar radiation, high temperatures, high electrolyte concentration, low nutrient availability and relative humidity ([60]; [88]). Such rocks are primarily colonized by stress-tolerant species ([60]). These species may obtain energy sources from precipitations, aeolian or aqueous organic matter inputs, and also from atmospheric trace gases deposition (e. g., CO₂, H₂, CH₄) ([50]). Chemolithotrophs are known to be the dominant groups of the bacterial community in young volcanic deposits ([50]). Previous studies have investigated the pioneering communities of bacteria colonizing freshly deposited volcanic ash ([36]; [71]) and highlighted their significant role in the fixation of carbon and nitrogen from the atmosphere ([50]; [51]; [52]; [55]; [71]; [72]). However, far too little attention has been paid to the colonization of the freshly deposited unvegetated tephra by fungal communities, which is also an important group of

pioneering microorganisms ([22]).

Fungi have been reported to be major components of soil microbial biomass ([4]), playing a pivotal role in the decomposition of organic matter ([94]) and controlling the rates of key ecosystem processes, such as fluxes of nutrients and energy ([66]). In addition, the symbiotic associations – mycorrhiza – that fungi can form with most plants (in particular conifers) are particularly adapted for the colonization of silicate minerals ([20]) and also of tephra ([74]). Through growth at the surface of minerals, fungi are able to acquire substantial amounts of inorganic nutrients such as K, P, Mg or Fe from these minerals ([19]; [79]) and, in doing so, to contribute to mineral weathering and soil formation. Thus, fungi are a pioneering group of microorganisms in these young mineral soils ([42]), likely driving the nutrient acquisition and their accumulation into the biological compartment of these new ecosystems.

Fungal hyphae can also represent a transport vector for other microorganisms (i. e. the so-called ‘fungal highway’) ([54]). The tephra invasion by fungi may lead to the redistribution of the bacterial and archaeal communities throughout volcanic soil profile ([13]). Previous studies have mainly used the fruiting body distribution to monitor the fungal colonization of volcanic tephra ([22]). Only few reports relying on cultivation-based and molecular methods have investigated the microbial community response to tephra deposition ([42]). However, to our knowledge, no research so far has been conducted on the distribution, diversity and composition of fungal vegetative body (i. e. mycelium) in freshly-deposited, unvegetated tephra.

The identification of soil fungal communities, assessment of hyphal growth and fungi interactions with rock-forming minerals have been studied using a wide range of culture-based approaches such as axenic or soil microcosms ([3]; [80]) or rhizotron setup ([15]). In-situ detection of fungi in soils is traditionally done by fluorescence staining

([17]), while biochemical characterization and biomass quantification are performed by analysis of fatty acid ([53]) or by quantifying membrane sterol ([70]; [59]). With respect to fungal weathering, it has been mostly investigated experimentally by scanning electron microscopy (SEM) (e.g., [47]), transmission electron microscopy (e.g., [10]) and more recently by synchrotron radiation techniques ([18]) or atomic force microscopy ([38]).

Although these techniques are effective in providing an overview of the fungal community composition and their effect on minerals at the sub-micron-scale, they have limitations. For instance, cultivation methods result in a restricted set of identifiable, cultivable fungal species ([3]; [6]). It is estimated that less than 20% of all known fungal species are cultivable on synthetic media under laboratory cultivation conditions. Besides, it is complicated to isolate Ectomycorrhizal (ECM) fungi in axenic culture ([28]), which drastically limit the diversity of fungi studied and their interactions with soil minerals. Phospholipid fatty acid (PLFA) 18:2w6, which is used as a marker for fungi ([44]), is also common in plants and animals ([53]). Although it may be easy to discriminate the plant roots from fungal hyphae because of their larger size, there is still a risk to overestimate the Fungal biomass (FB) due to the presence of smaller, sometimes, not visible plant residues. Thus, fungal biomass measurement by this technique is not systematically reliable.

Techniques of estimating of fungal biomass through signal molecules such as ergosterol are being applied ([70]; [59]), but might be limited by the destruction of hyphae during sample homogenisation, environmental conditions, sample storage and natural variability of fungal communities ([70]; [21]). For instance, exposure of the sample to short wavelength UV radiation induces ergosterol degradation due to photochemical oxidation ([58]). Temperature also affects the fate of ergosterol in sample: ergosterol degradation was shown to decrease by 60% within 3-6 months of sample incubation at 15 °C compared to 25 °C

([8]). Long storage of soils in the laboratory conditions (without plants growing on it) may lower the content of ECM fungi that lack carbon source from their host-plant ([90]; [34]). Moreover, ergosterol, which was assumed to be readily degraded after the fungal death, was used extensively as a marker of living fungi (e. g., [70]; [59]). However, decay of ergosterol from fresh fungal necromass in soil is, in fact, slow ([58]). As a result, ergosterol might be useful solely as an indicator of fungal presence, but not for quantitative FB determination ([95]). In order to minimize the above-mentioned issues, sampling of soils for ergosterol was suggested to be made preferentially either in autumn when higher proportion of living fungal biomass, especially ectomycorrhizal mycelium ([8]; [91]), is presumably present, or during winter because of the limited plant-resource competition with bacteria, high moisture and generally mild temperatures of the Mediterranean winters ([94]).

The ergosterol content of a given soil can also vary from one ecosystem to another as a result of fundamental variations in the composition of fungal community, which itself is affected by soil pH, the season, the organic matter quality, the presence of various fungal groups synthesizing different amounts of ergosterol per biomass unit ([70]). Average ergosterol-to-fungal biomass ratio varies from 2.2 to 24 mg ergosterol per g of FB ([29]). In tephra deposits, the quantitative extent of fungal colonization has been very seldom investigated (and the ergosterol method rarely used in this context) ([42]) as most studies rather focused on the bacterial and fungal communities structure and diversity.

For this study, our aims were to characterize the pioneering communities of microorganisms colonizing freshly-deposited tephra from Mt. Etna eruption (on November 23, 2013) over a 2-year period. With respect to previous studies, our focus is more on the fungal biomass and community assembly as those microorganisms are effective agents of mineral weathering and can contribute actively to soil formation and thus help re-vegetation. Estimation of FB was primarily done via membrane sterol extraction and purification, while the traditional method

of nucleic acid extraction and Denaturing gradient gel electrophoresis analysis (DGGE) was applied to characterize tephra and soil fungal community composition. Further, purification and amplification by Polymerase chain reaction (PCR) linked to a clone sequence based analysis or a community fingerprinting were also performed. To complement our molecular approach, we also used SEM and fluorescence-based analysis to visualize hypha-mineral interaction.

4.2 MATERIALS AND METHODS

4.2.1 *Study site*

Mt. Etna (Italy) is a 3300 m high basaltic volcano in eastern part of Sicily in the middle of the Mediterranean Sea covering an area of about 1250 km² ([5]; [26]). Mt. Etna is characterized by permanent degassing and paroxysmal effusive and explosive activity, mainly concentrated at the summit craters. Because of the dominant wind direction, pyroclastic fall deposits occur mostly on the northeast, east and southeast flanks of Etna ([1]). Our sampling site is located in northeastern part of the volcano (Fig. 4.1) near Mt. Sartorius, in an undisturbed portion of a coniferous forest at an altitude of 1755 m above sea level (a.s.l.). In this area, the soil is formed on basaltic volcanic material. However pedogenesis was interrupted by several tephra deposition events, the most recent being on November 23, 2013, which resulted in the fallout of 20 cm thick layer of tephra (> 2 mm) ([16]).

The climate of Mt. Etna is distinct from that in the other parts of Sicily due to altitude, slope and local exposure on the volcano. According to Monte Conca weather station located at 1820 m a.s.l. on the Etna north side close to our sampling site, January is the coldest month (1 °C), July and August are the warmest (18 °C). Most of rain occurs on the eastern part of Mt. Etna due to the slope exposure to the dominant

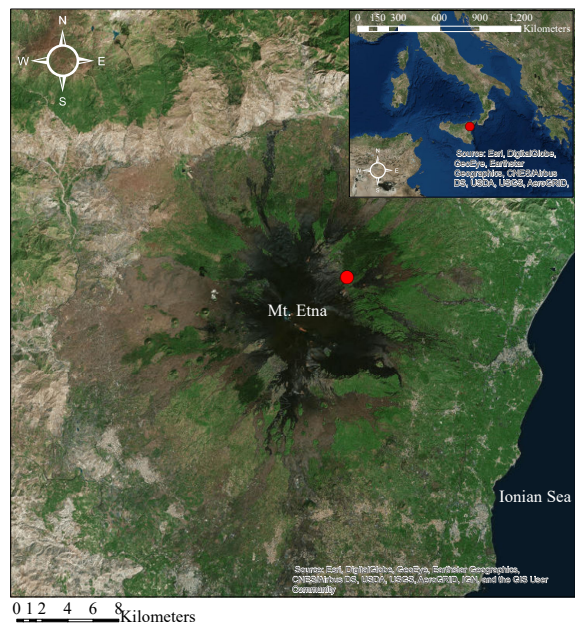


Figure 4.1: Map of the study sites in 2014 and 2015. Red circle - sampling site (GPS coordinates for sampling site in 2014 : UTM x=0504020, y=4181577; GPS coordinates for sampling site in 2015: UTM x = 0504050, y = 4181572).

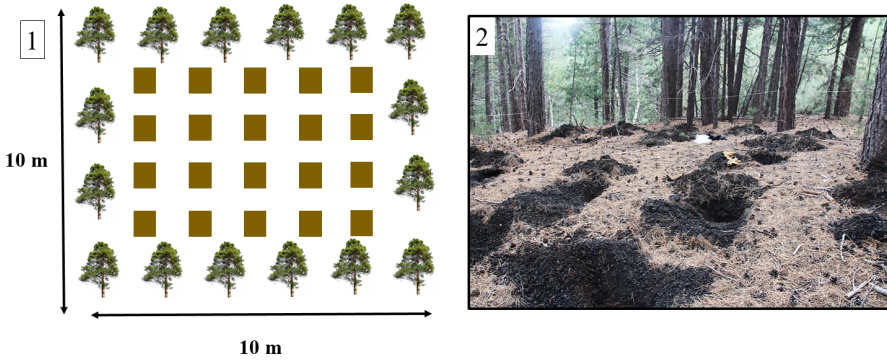


Figure 4.2: Schematic representation of sampling site and procedure (1). Size of the plot (10×10 m) and distribution of soil profiles (2 m) sampled. The samples of the various tephra (>2 mm, TT, BT, and <2 mm, T) and 'buried' soils (S1, S2 and S3) were collected up to 40 cm depth. The sampling was performed annually in September 2014 and 2015. Twenty soil profiles were dug within a 10×10 m plot. Composite samples were made of material collected from five soil profiles located at distance of 2 m from each other. In total, four composite field replicates were sampled from six tephra and soil layers, resulting in 24 samples. Sampling site photo: Sicily, Etna (2). Coniferous forest. Elevation: 1755 m above-sea-level.

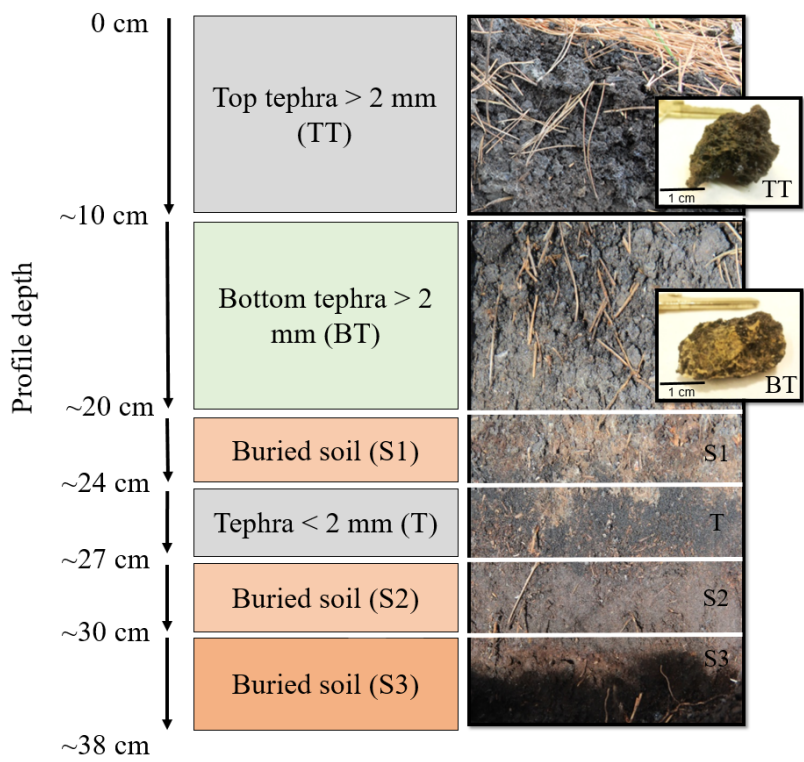


Figure 4.3: Schematic representation and photo of the volcanic soil profile, where TT (top 10 cm) + BT (bottom 10 cm) is the tephra deposited on November 23, 2013. *The colours used here to illustrate the different layers in volcanic soil are kept in Section 4.3. The soil horizons have the same characteristics in all sites.

winds. Thirty-year averaged data (1965-1994) show that the amount of rain changes from 400 mm on the lower SW slope up to 1200 mm at 600-700 m elevation on the eastern slope, with a mean value of about 800 mm ([26]). Maximum precipitation is in the autumn-winter season (mostly in October) while the minimum is always in July (generally no precipitation). At high elevation (> 2000 m), snow represents a signifi-

cant part of the precipitation for most of the year (October-April) ([26]).

The sites for 2014 and 2015 sampling campaigns were located 10-15 m from each other to avoid any discrepancy in environmental conditions. The sampling was performed at the same altitude each year on slopes of less than 10 deg. The study site was located in the coniferous forest and was surrounded by *Pinus nigra* subsp. *laricio* established before the Mt. Etna eruption (Fig. 4.2). No herbaceous species or shrubs appeared in the following two years after tephra deposition. The 20 cm layer of freshly deposited tephra (i. e. trachy-basaltic scoria of > 2 mm in diameter) could be divided into a topmost section of 10 cm thick dark coloured tephra (TT) and a 10 cm thick bottom tephra layer (BT), which was characterized by abundant yellow/white incrustation. The freshly-deposited tephra overlayed a 4 cm thick organic-rich soil layer (S1) intermixed with fine-grained tephra (see Figure 4.3). This S1 layer contained undecomposed fine roots and small branches and overlayed a 3 cm thick trachy-basaltic volcanic ash (< 2 mm) deposit of unknown age (T). A second organic-rich soil layer (S2) was buried under the T deposit and also contained poorly decomposed branches and fine roots. Deeper, a more developed 8-cm thick loamy-sand soil layer (S3) was formed on tephra (or scoria > 2 mm) deposit, this horizon was wetter compared to the overlying soil and without roots or branches.

4.2.2 Sampling strategy

The samples of the various tephra (TT, BT > 2 mm, and T < 2 mm) and 'buried' soils (S1, S2 and S3) were collected up to 40 cm depth. The sampling was performed annually in September 2014 and 2015. Twenty soil profiles were dug within a 10 × 10 m plot (Fig. 4.2). Composite samples were made of material collected from five soil profiles located at distance of 2 m from each other. In total, four composite field replicates were sampled from six tephra and soil layers, resulting

in 24 samples.

To prepare samples for further analyses, visible plant residues and soil fauna were removed. The tephra and soil were air-dried for 1 month (in thin layers in open wooden box) and then soil was sieved to < 2 mm and ground with pestle and mortar before being stored in plastic bags at the room temperature. The tephra samples were also ground by the same procedure.

For ergosterol extraction and molecular analysis, fresh soil samples were sieved through 2 mm diameter mesh. Tephra samples were ground with pestle and mortar. After homogenization, samples were kept at -20 °C. The water content was calculated by drying 1 g of tephra and soil material at 105 °C during 24 hours.

All tephra and soil sample analyses were performed in 2014 and 2015. Long-term sampling strategy was repeated in the same season each year to avoid the possible effect of environmental variability on the results.

4.2.3 *Tephra and soil characterization*

In order to determine the chemical composition of tephra and soil samples, total digestion was performed by lithium metaborate and tetraborate fusion at 1000 °C and the subsequent fusion bead was recovered in HNO_3 1N ([24]). The solution was measured by ICP-AES ($< 3\%$ uncertainty relative to the basaltic standard material BHVO-2 of the USGS). The Loss on ignition (LI) was assessed at 1000 °C and the elemental content of the tephra and soil was normalized to dry weight at 105 °C, which was then used to derive the Total reserve in bases (TRB) like in Eq. (4.1). TRB represents the sum of the alkaline and alkaline-earth cations (Ca^{2+} , Mg^{2+} , K^+ and Na^+ , cmolc kg^{-1}) and can be used to compare soils evolved from similar lithology as it is highly dependent

on rock-forming minerals. Generally, TRB values in soils are lower than in the underlying bedrock.

$$\text{TRB} \left[\frac{\text{cmolc}}{\text{kg tephra/soil}} \right] = [\text{Ca}^{2+}] + [\text{Na}^+] + [\text{K}^+] + [\text{Mg}^{2+}] \quad (4.1)$$

The pH in tephra and soil samples was measured in demineralized H₂O in 1:5 tephra/soil: water ratio ([65]). Total organic carbon (TOC) and Total nitrogen (TN) were determined with a Vario Max dry combustion CN analyser instrument (Elementar Analyses system GmbH, Germany) within 1 g of soil (< 2 mm) or crushed tephra. Total organic matter (TOM) was estimated by the LI, or gravimetric method ([9]) according to Eq. (4.2):

$$\text{TOM}[\%] = \frac{\text{Wt. } 105^\circ\text{C} - \text{Wt. } 550^\circ\text{C}}{\text{Wt. } 105^\circ\text{C} - \text{Wt. crucible}} \times 100 \% \quad (4.2)$$

where Wt. 105 °C/550 °C is the mass of air-dried crushed tephra or ground soil fraction (usually 1 g) after drying at 105 °C and after ignition in muffle furnace at 550 °C both for 24h. The weighing after ignition was done after cooling the samples in a desiccator.

4.2.4 Imaging of tephra

Fluorescence microscopy

In order to image fungal filaments on the surface or inside tephra particle, we used a fluorescence microscope (Leica DMRXA) with a filter combination of 460-500 nm for excitation, dichroic mirror 505 nm and 512-542 nm bandpass for emission filter. The fluorescent dye, Fluorescein isothiocyanate (FITC), conjugated the lectin Wheat germ agglutinin (WGA), which is highly specific for chitin, a polymer of N-acetylglucosamine (GlcNAc) ([17]). Chitin is a major characteristic structural component of fungal cell walls ([17]; [92]) and, thus, this staining method provides an efficient mean to visualize the fungal filament in soil and rock matrices.

Briefly, the fungi visualization was performed as following: the mycelium attached to the tephra surface was removed from the particles collected in 2014 with tweezers and placed in the eppendorf tubes to react with [WGA-FITC](#) dye solution (100 $\mu\text{g}/\text{ml}$ [WGA-FITC](#) in 0.1 M-HEPES buffer at pH 7.8) for 30 min. The tubes were then centrifuged and the pellets were rinsed with 1 mL of 0.1 M HEPES buffer at pH 7.8 and the flasks were centrifuged again. This procedure was repeated three times to rinse out the dye. After a final centrifugation, filaments were resuspended and a drop was deposited on the glass slide and observed with the fluorescence microscope.

To visualize the presence of filaments within tephra collected in 2014, air-dried single particles of ~ 2 cm in diameter were vacuum-embedded with epoxy resin (EpoFix-Struers) and let to cure for a week, then cut with a low speed precision diamond saw in sections of ~ 4 mm thick, which were later polished. The thin sections were treated with [WGA-FITC](#) dye solution for 30 min, then rinsed out thoroughly with 0.1 M-HEPES buffer at pH 7.8 and observed as mentioned above. Sections and fungal crust were checked before [WGA-FITC](#) treatments to detect the autofluorescent region and feature in the observed polished slice of tephra. Generally, auto-fluorescence was low in all the thin sections and fungal filaments.

Scanning Electron Microscopy

To analyse the fungal surfaces and detect the presence of hyphae within tephra vesicular structures, particles collected in the TT layer (0-10 cm) and in the BT layer (10-20 cm) in 2014 were air-dried and then coated with chromium by using Cressington evaporator for [SEM](#) (FEG-SEM JEOL JSM-7600F). Thin sections of tephra particles (fabricated as mentioned above in the previous paragraph) were imaged on Hitachi SU 70 operating at a voltage of 20 kV, working distance of ~ 15 mm.

4.2.5 *Quantification of fungal biomass: ergosterol extraction and HPLC analysis*

Ergosterol concentrations were measured using the microwave-assisted extraction technique modified according to [59]. Four field replicates sampled in 2015 were used for ergosterol extraction. Briefly, sieved soil (1 g fresh weight) and crushed tephra (2 g fresh weight) samples were placed into Teflon tubes. The tephra and soil samples were mixed, respectively, with 0.05 or 0.5 mL of an internal standard (i. e. 200 ppm cholesterol) and 2 mL of 0.5 M NaOH-methanol solution. Cholesterol was added to blank, calibration standards, tephra and soil samples in order to account for the potential losses of ergosterol during sample preparation and transfer. The tubes were tightly closed with Teflon screw caps and saponified twice by heating in the microwave (Ultra-WAVE). During the first heating, the microwave instrument was operated at 1500 W at 130 °C at 40 bar nitrogen pressure for 18 s. The second heating was performed at 100 °C during 17 s. after 15 minutes of sample cooling. After cooling down, solutions and solid samples were transferred to the glass tubes. 1 mL of distilled water was added to the Teflon tubes to mobilize samples retained on the bottom and then also transferred to the glass tubes. The contents were extracted with 2 mL of High-performance liquid chromatography (HPLC)-grade pentane (added to the Teflon tubes and then transferred to the glass tubes) to separate the phases. The tubes were vigorously vortexed for 5 min and then centrifuged at 10000 g for 1 min. The upper pentane phase was removed and transferred to the new tubes. This procedure was repeated three times and the pentane extracts were combined for further analysis. The extracts were evaporated under the stream of nitrogen gas at 35 °C to minimize oxidation of the extracted ergosterol and then were redissolved in 2.5 mL of HPLC-grade methanol (soil samples) and in 0.5 mL of methanol (tephra samples). 50 µL of the extracts were injected for ergosterol concentration measurements into HPLC Agilent 1200 equipped with a degasser, a quaternary pump, an automatic injector and a diode-array detector (DAD). The HPLC was

fitted out with LichroCART® 100 RP18e 125 mm by 4 mm analytical column with a particle size of 5 μm . Acetonitrile-methanol solution (98:2, v/v) was used for isocratic elution of the extracts at a flow rate of 2.0 mL min^{-1} at 40 °C. Ergosterol and cholesterol peaks were identified with the ultraviolet (UV) detector set at 282 nm and 210 nm, respectively. The retention time of ergosterol and cholesterol was 5.3 and 7.7 minutes, respectively. Concentrations of ergosterol in samples were quantified by comparison with standard solutions (based on ergosterol: internal standard ratio). The limit of detection (LOD) was determined at 0.05 $\mu\text{g g}^{-1}$ substrate and the limit of quantification (LOQ) was determined at 0.13 $\mu\text{g g}^{-1}$ substrate.

Estimation of potential losses of ergosterol during the extraction procedure was quantified by the recovery of 10 ppm ergosterol in the blank sample. The efficiency test of ergosterol extraction was determined by spiking the soil with undetectable ergosterol concentration with 20 ppm of ergosterol. This extraction efficiency of ergosterol was estimated at ~ 95%. Fungal biomass was assessed based on average ergosterol content of 5.1 mg g^{-1} in the FB ([27]). The FB was calculated using Eq. (4.3):

$$\text{FB} \left[\frac{\mu\text{g}}{\text{g}} \right] = \text{Erg} \left[\frac{\mu\text{g}}{\text{g}} \right] \times f \times R \quad (4.3)$$

where FB is the fungal biomass (μg) and Erg is the extracted ergosterol (μg), both in ~ 1 g Dry weight (DW) crushed tephra or < 2 mm soil fraction. Soil and tephra dry weight was estimated by reference to oven-dried replicates at 105 °C; f is the conversion factor, $f = 196$ ($1/5.1 \text{ mg g}^{-1} \times 1000$, $\text{mg biomass } \mu\text{g}^{-1} \text{ ergosterol}$); R is the correction factor for extraction recovery, $R = 1.05$ (100%/ extraction efficiency 95%).

4.2.6 Molecular analyses

DNA extraction

All tephra layers and soil samples were subjected to Deoxyribonucleic acid (DNA) extraction in order to assess fungal community composition in soil profiles after tephra deposition. Two field replicates collected in 2014 and four field replicates sampled in 2015 were used for DNA extraction, resulting in 12 and 24 samples, respectively, in total. Briefly, DNA of 0.25 g of crushed tephra or < 2 mm soil fraction were extracted using Mo Bio's PowerSoil DNA Isolation Kit (Qiagen, Mo Bio Laboratories, Carlsbad, CA), according to the manufacturer's recommendations. The quality and the amount of DNA extracted from the tephra and soil samples were assessed by electrophoresis on agarose gel (1% w/v in TAE 1× Tris Acetate EDTA buffer) at a voltage of 100 V. DNA samples (15 µL DNA extract mixed with 3 µL of loading dye) were loaded onto the agarose gel alongside DNA markers for approximate size determination and nucleic acid quantification (O'GeneRuler 1 kb Plus DNA Ladder, Thermo Fischer Scientific). To overcome the toxicity problem of ethidium bromide, gels were stained with the non-toxic and non-mutagenic GelRed (Biotium, Hayward, CA, USA; [46]) and visualized under UV illumination. The lower detection limit of GelRed is around 0.1 ng DNA per DNA band present in the gel.

DNA quantification

DNA was quantified based on the 1D gel electrophoresis image analysis software GelAnalyzer¹ and analysis of densitometry profiles of GelRed-stained DNA gels. Two field replicates collected in 2014 and four field replicates sampled in 2015 were used for DNA evaluation (Figs. 4.4a-d). This latter figure presents DNA gel electrophoresis for selected samples collected in 2014 and 2015. Using GelAnalyzer 2010, densitometry profile for each lane was analysed and compared to the

¹ 2010, <http://www.gelanalyzer.com/> by Istvan Lazar

profile of DNA ladder of known quantities of nucleic acids (0.1 µg per µL DNA ladder) (Fig. 4.4e). From the densitometry analysis of DNA ladder, the total intensity area per ng DNA added in the gel was computed for each gel. The determination of DNA amount in each sample is based on the conversion of total intensity area of each profile of DNA sample into ng DNA per µL DNA sample loaded in the gel using the average conversion factor from DNA ladder profiles (e. g., 38 A.U. (intensity area) / ng DNA).

PCR amplification of fungal DNA

The primer pair GC-clamp-Myers-Internal transcribed spacer (ITS)1 (59 bp; the 40-mer GC-clamp is attached to the 5'-end of the forward primer):

CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCG
CCCC – TCCGTAGGTGAACCTGCGG

([93]) and ITS4-KY01 (20 bp):

TCCTCCGCTTWTGWTGTC

were used to target the ITS region of the fungal, nuclear ribosomal RNA genes, one of the most commonly used phylogenetic marker genes for microbial ecology studies.

The sequence of the GC clamp is based on the sequence published by [77] with small modifications as described by [63] and [61]:

CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCC.

The ITS1 sequence (5'-TCCGTAGGTGAACCTGCGG-3') hybridizes to the 18S rRNA gene upstream of the ITS1 region. The ITS4-KY01 sequence (5'-TCCTCCGCTTWTGWTGTC-3') hybridizes to the 28S rRNA gene downstream of the ITS2 region ([83]; [14]).

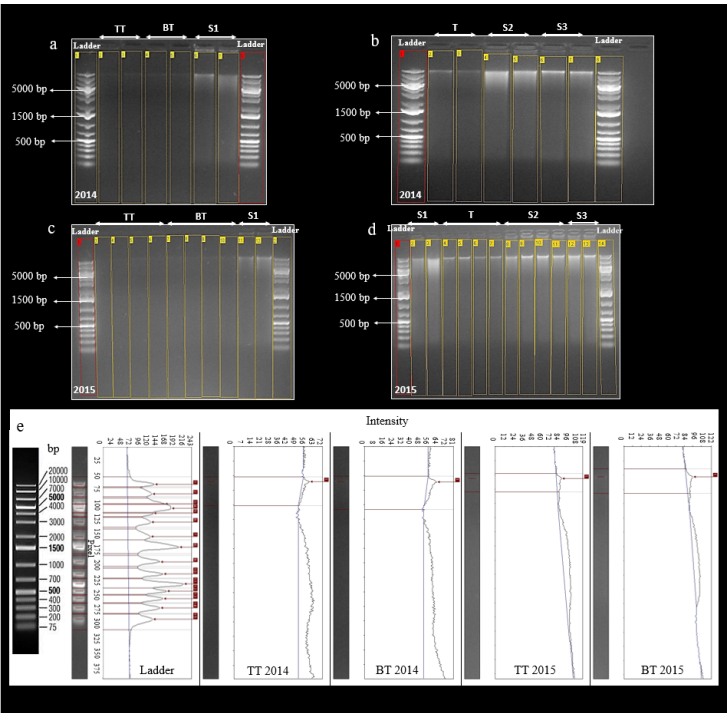


Figure 4.4: O'GeneRuler 1 kb Plus DNA Ladders are on the left and right-end side of each gel image (a-d). In between the ladders, each lane represents individual replicates within each horizon -labels on top- for 2014 and 2015. Images of gel were analysed by Gel-Analyzer software. In Figure 4.4e, the profile windows show the intensity of the DNA ladder and TT and BT samples from 2014 and 2015 (blue line is the background level). The vertical axis in Fig. 4.4e is the distance from the lane's start position in pixels, while the horizontal axis shows the intensity values. The ladder on the left of Fig 4.4e is the reference lane image from supplier of O'GeneRuler 1 kb Plus DNA Ladder (in base pair - bp).

Fungal ITS1-ITS2 sequences were amplified in a 50 μL -PCR mix containing 0.25 mM deoxyribonucleotide triphosphate mix (dNTPs), 0.2 μM of each primer, 10 μL of 5 Herculanase II reaction buffer (Agilent Technologies, Santa Clara, CA), 0.5 μL of Herculanase II fusion DNA polymerase and a specific volume of DNA samples (4 μL of tephra-derived DNA or 2 μL of soil-derived DNA samples). The Herculanase II reaction buffer provides a final Mg^{2+} concentration of 2 mM. Negative controls include PCR amplification reactions with 2 μL of Diethyl pyrocarbonate (DEPC)-treated water.

The PCR was performed with a Biometra TGradient cycler (Biometra, Goettingen, Germany) under the following conditions: thermo cycler set at 98 °C before insertion of PCR tubes (simplified hot start method), 98 °C for 2 min, 35 cycles (98 °C for 20 s, 47 °C for 20 s, 72 °C for 30 s) and a final extension at 72 °C for 30 min. The size of the PCR amplicons was assessed by electrophoresis on agarose gel (1% w/v in TAE 1 $\times$ (Tris Acetate Ethylenediaminetetraacetic acid (EDTA) buffer) at a voltage of 100 V. The PCR amplicon samples (5 μL mixed with 1 μL of loading dye) were loaded onto the agarose gel alongside DNA markers for approximate size determination (O'GeneRuler 1 kb Plus DNA Ladder, Thermo Fischer Scientific).

DGGE of PCR fungal DNA products

PCR amplicons were separated on the basis of the GC contents. DGGE analysis was carried out with the DCode universal mutation detection system (Bio-Rad, Hercules, CA). PCR products were loaded onto 6% (w/v) polyacrylamide (37.5:1 acrylamide:bisacrylamide, Sigma) gels with a linear denaturing gradient from 35 to 65% (for samples from 2014) and from 25 to 50 (for samples from 2015) (where 100% contains 5.6 M urea and 32% (v/v) deionized formamide) in 1 TAE buffer (20 mM Tris base, 10 mM acetate, 0.5 mM EDTA [pH 8]). The electrophoresis was run at 60 °C and 100 V (for samples from 2014) and 120V (for

samples from 2015) for 16 h. Gels were stained with SybrGold (Invitrogen) and photographed under a UV transilluminator.

Sequencing of DGGE fungal bands

Two methods, i. e. **DGGE** sequencing and ribotypes counting, were applied for the estimation of fungal community assembly. With 2014 samples (including sample from TT to S3), the quality of **DGGE** gel was not optimal (data not shown) nevertheless a few bands were exploitable for **DNA** sequencing and species identification. For 2015 samples (including samples from TT to S3), **DGGE** gels show clear and separate bands that were used for ribotype counting.

The sequencing of **DGGE** bands was done as follows. Bands of interest were excised from the gel using a sterile blade and incubated overnight at 4 °C in DEPC-treated water. This allowed for **DNA** diffusion out of the polyacrylamide matrix. The eluted **DNA** was diluted 10 times with DEPC-treated water, mixed and amplified using the same primer pair without the GC clamp (ITS1 and ITS4-KY01). The **PCR** products earmarked for sequencing were purified using the UltraClean **PCR** Clean-Up Kit (MO BIO Laboratories, Inc, 2746 Loker Ave West, Carlsbad, CA 92010). The purified **PCR** product was then sequenced at Macrogen Europe (ISV Institute, Amsterdam, The Netherlands) using an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA) with the primers ITS1 and ITS4-KY01. The sequencing data were edited with the Geneious Pro R6 software (version 6.1.4, Biomatters Ltd., Auckland, New Zealand). A BLASTN search against the non-redundant (nr) database was carried out in Geneious 6.1.4 using the default parameters.

The ribotype counting was performed directly on the gel images using a GelAnalyser 2010 software ([2]; [21]). The procedure explained above in this section was carried out and the number of detected bands was counted.

4.2.7 Statistical analysis

Using R-studio software, linear Bivariate least square regression (BLS) technique was used to determine statistical significance of differences in values measured for pH, TOM, TOC, TN, Water content (WC), DNA and FB between sampling years. Intercept with axes different from zero would indicate a systematic analytical bias between the years while a slope different from unity would reflect a proportional bias ([33]). The null hypothesis suggests the equivalence and the alternative hypothesis suggests a bias between the years. If the null hypothesis is rejected, the two sampling years are considered as not equivalent. If it is not rejected, equivalence is not proven, but assessed using a confidence interval approach (CI = 95%). The size of the interval is directly linked to the random measurement errors and the sample size. Significance was defined at $p < 0.05$.

Pairwise comparison using Wilcoxon rank sum test was used to estimate the significance of differences in values obtained for pH, TOM, TOC, TN, WC, DNA and FB between the layers (TT, BT, T, S1 and S2) in both years. Significance was defined at $p < 0.05$.

DGGE data were converted to binary format using the Jaccard index and presented as a proximity matrix ([48]; [21]). Jaccard index defines similarity of each sample pair based on the number of DGGE bands existing in one or more fungal communities. DGGE bands do not contribute to the similarity of the communities if they are not presented in either of two comparable communities. The Jaccard index was calculated using Eq. (4.4):

$$\text{Jaccard index} = \frac{\text{the number of bands in either sample}}{\text{the number of bands in both samples}} \quad (4.4)$$

4.3 RESULTS

4.3.1 *Tephra and soil general properties*

According to the Total alkali-silica (TAS) classification, both fresh (TT, BT) and 'buried' tephra (T) samples can be referred to as trachy-basalt, which is characteristic of Mt. Etna volcanic material ([87]; [40]). Figure 4.5a shows a clear difference in TRB values between tephra (TT, BT and T) and soil (S1, S2 and S3) layers with tephra presenting higher TRB values (ranging from 727 to 846 cmolc kg^{-1}) than soil samples (TRB values between 514 and 655 cmolc kg^{-1}). Between 2014 and 2015, TRB profiles did not change significantly. The pH values of the soil samples (S1, S2 and S3) ranged from 5.5 to 5.9 in 2014 and from 5.2 to 5.8 in 2015 (Figs. 4.5b and C.1; Table 4.1). In tephra (TT, BT and T), pH was more alkaline: 6.1 - 6.7 in 2014 and 5.8 - 6.6 in 2015. In comparison, the fresh tephra collected one day after the eruption had a pH of 8.9.

TOC, TN and TOM contents over the entire profile did not vary significantly between 2014 and 2015 ($p = 0.31$, $p = 0.67$, $p = 0.34$, respectively) however it was noted that TOC, TN and TOM were significantly lower in the tephra samples (TT, BT and T) compared to soil samples (S1, S2 and S3) (Table 4.1; Fig. C.1). TOC content of the soil layers ranged from 27.2 to 114.8 g kg^{-1} in 2014 and from 39.5 to 113.4 g kg^{-1} in 2015 (Table 4.1; Fig. 4.5c) decreasing with depth. The tephra TOC content ranged from 0.6 to 6.4 g kg^{-1} and from 0.7 to 5.8 g kg^{-1} in 2014 and 2015, respectively, increasing with depth. TN values of tephra (TT, BT, T) varied from 0.1 to 0.3 g kg^{-1} in both years, while TN in the soil samples (S1, S2, S3) ranged from 1.1 to 3.8 g kg^{-1} in 2014 and from 1.1 to 3.4 g kg^{-1} in 2015, decreasing with depth (Fig. 4.5d, Table 4.1). No significant difference in TN values for tephra and soil was found between the years. Values of TOM in soils (S1, S2 and S3) decreased with depth varying from 69.8 to 246.6 g kg^{-1} in 2014 and from 79.0 to 249.6 g kg^{-1} in 2015. TOM in tephra (TT, BT and T) ranged from 0 to 12 g kg^{-1} in both years and tended to increase with depth, however

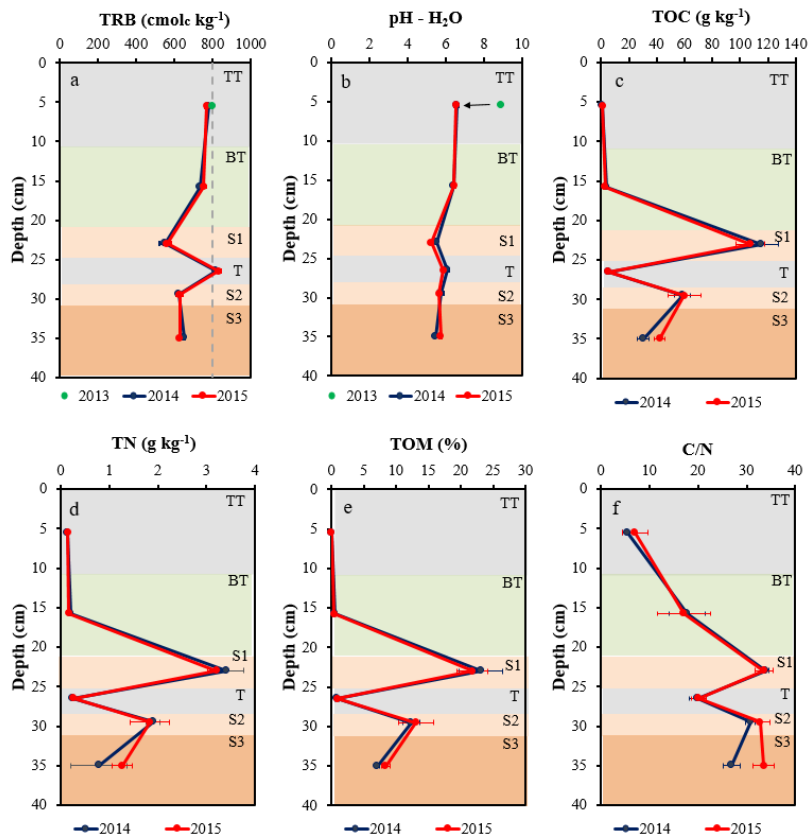


Figure 4.5: Characterization of the tephra and soil (<2 mm fraction) samples: total reserve in bases (TRB, cmolc kg⁻¹) (a). Dotted vertical line represents the TRB value of the fresh tephra deposited on November 23, 2013; soil acidity (pH-H₂O) (b); total organic carbon (TOC, g kg⁻¹) (c); total nitrogen (TN, g kg⁻¹) (d); total organic matter (TOM, %) (e), The ratio of total organic carbon to total nitrogen (C/N) (f).

it was always lower than TOM in soil layers (Fig. 4.5e, Table 4.1). The TOC to TN ratio (C/N) of the soil layers ranged from 29.8 to 34.6 in

2014 and from 31.0 to 35.5 in 2015 (Fig. 4.5f, Table 4.1). The tephra C/N ratio ranged from 4.5 to 22.3 and from 5.2 to 23.2 in 2014 and 2015, respectively, being always higher in BT than in TT.

Water contents (WC) were significantly lower in the tephra compared to soil layers (Fig. C.2, Table 4.1). At the exception of BT sample from 2015, which exhibits a WC of 6.4%, the rest of the tephra values ranged from 0 to 1.7% in 2014 and to 2.8% in 2015. In S1, S2 and S3 layers collected in 2014, WC varied from 1.8 to 2.9%, while in 2015 the range was laying in between 6.5 and 28.8%.

4.3.2 Fungal colonization of freshly-deposited tephra

The colonization of the surface of tephra particles from the BT was readily visible under the form of yellowish crust that developed within 10 months of deposition (Figs. 4.6, 4.6a insert). Although not uniform over single particles and not systematically-present on each tephra particle of BT, the encrustation is large enough to change the colouration of the BT layer and to distinguish it clearly from TT in the field (Fig. 4.3).

The nature of these crusts is fungal as evidenced by the positive WGA-FITC staining obtained from the surface of a 2014 BT particles (Fig. 4.6a). This biological crust is formed by a dense network of interconnected hypha (Figs. 4.6d and 4.6e). Detailed electron micrographs of the surface of BT tephra showed that some individual hypha are forming fungal structure resembling an appressorium – a specialized fungal cell dedicated to breakthrough host-plant cells, - which is physically penetrating the surface of tephra particle (Fig. 4.6c). In contrast, for TT particle, despite the presence of individual hypha within tephra particle (Fig. 4.6b), the surface was overall devoid of fungal mycelium emphasizing the fact that the fungal colonization of the topmost tephra layer was minimal (Fig. 4.6f).

Fungal biomass

The upper layers, TT and BT, tend to show a slight increase in fungal biomass (via ergosterol extraction) between 2014 and 2015. In TT, the fungal content increased from $134 \pm 11 \mu\text{g g}^{-1} \text{ DW}$ in 2014, to $151 \pm 49 \mu\text{g g}^{-1} \text{ DW}$ in 2015. For BT, the **FB** increase was higher: from $363 \pm 205 \mu\text{g g}^{-1} \text{ DW}$ in 2014 to $565 \pm 344 \mu\text{g g}^{-1} \text{ DW}$ in 2015. The deeper layers (i.e. S1, T and S2) showed in general much higher **FB** than the upper TT and BT layers (Fig. C.2). For instance, S1 exhibited a large **FB** reaching $9775 \mu\text{g g}^{-1} \text{ DW}$ in 2014. However, this amount declined steeply in 2015 to $5346 \pm 572 \mu\text{g g}^{-1}$. Between 2014 and 2015, S1, T and S2 - the deeper layers - tended to lose **FB** in contrast to TT and BT, which have gained in **FB** (Fig. 4.7). However, it is important to bear in mind that amounts of **FB** detected in tephra and soil samples were highly variable illustrating the heterogeneous, non uniform character of fungal colonization. As a result, changes in biomass between 2014 and 2015 were difficult to interpret at this stage. *Sensu stricto*, the **FB** changes in all layers from 2014 to 2015 were not statistically significant ($p=0.0647$).

Molecular analyses

DNA EXTRACTION AND AMOUNT Figure 4.8 illustrates the **DNA** profile after gel electrophoresis obtained for tephra and soils layers in 2014 and 2015. Each extracted **DNA** sample shows an intense band at the top of the **DNA** profile corresponding to high molecular weight (HMW) **DNA** fragments ($> 10 \text{ kb}$). Below this band, a **DNA** smear occurred corresponding to double-stranded **DNA** molecules sheared at different levels during the **DNA** extraction procedure. The bands align on lanes that correspond to each soil and tephra layers. Each band corresponds to a set of **DNA** fragments of similar size (in base pair -bp) that can be estimated by comparing its position in the lane with respect to the ladder.

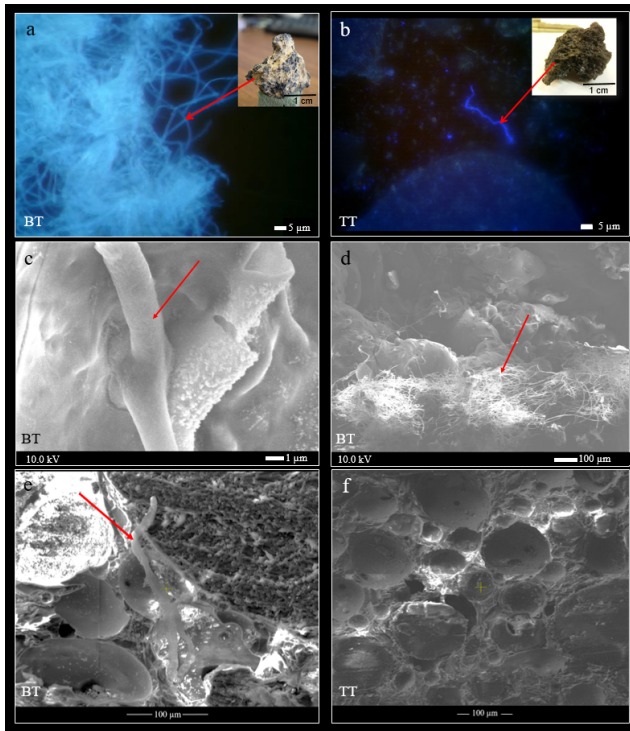


Figure 4.6: An insert: representative colonized tephra particle from the BT layer (~ 2.5 cm in diameter) showing large encrustation of fungal mycelium clearly visible in fluorescence microscopy by staining with WGA-FITC, a dye highly specific of chitin (a). In contrast, TT tephra particle surface (insert B) is devoid of fungal encrustation. A few hypha are present within vesicular tephra structure as evidenced by WGA-FITC staining (b). Note that both tephra were sampled in 2014. (c and d) Electron micrographs of the surface of BT tephra collected in 2014 showing extensive fungal mycelium (d) and a fungal structure penetrating the mineral substrate (c). Electron micrographs of the thin sections of BT (e) and TT (f) tephra collected in 2014 showing fungal colonization (e) and no evidence of fungal colonization (f).

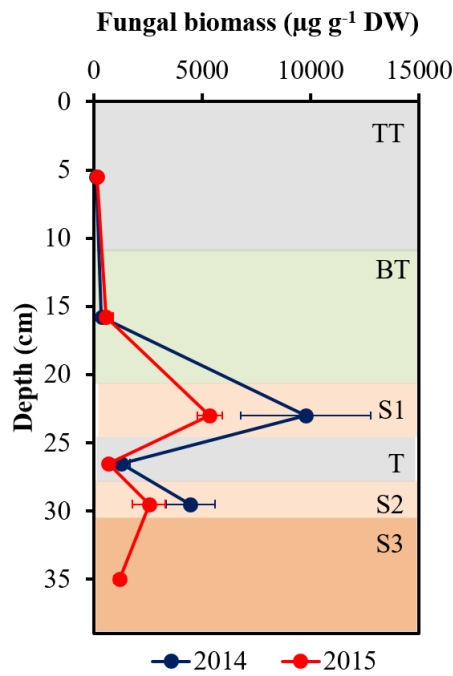


Figure 4.7: Fungal biomass (FB) profile with soil depth in 2014 and 2015. FB is the mean of four sample replicates. Error bars represent SD from the mean.

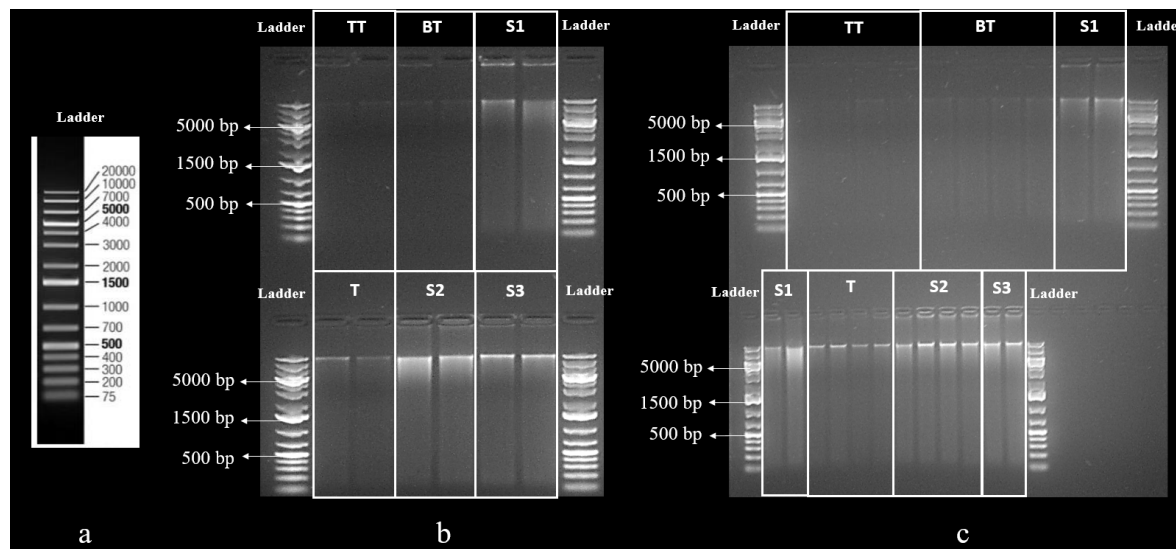


Figure 4.8: O'GeneRuler 1 kb Plus DNA Ladder representing the known quantities of nucleic acids (0.1 µg per µL DNA ladder) (a). This calibration DNA Ladder bands varies from 75 to 20000 bp. Note that the same ladder is present on the right and left-end side of each gel. Gels of DNA extracted from tephra and buried soils samples collected in 2014 and 2015 respectively (b and c). Lanes from left to right correspond to following replicates: TT, BT, S1, T, S2 and S3.

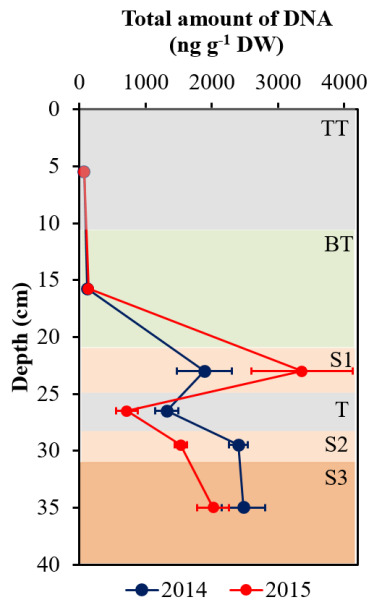


Figure 4.9: Total amount of extracted DNA (from both bacterial and fungal communities) quantified by the 1D gel electrophoresis image analysis software Gel-Analyzer and analysis of densitometry profiles of GelRed-stained DNA gels. Total amount of DNA is the mean of two sample replicates for 2014 and four sample replicates for 2015. Error bars represent SD from the mean.

From Figure 4.8, the near absence of visible bands for BT and TT indicates that the amount of DNA extracted was very low, both for 2014 and 2015. Indeed, the amount of extracted DNA from TT layer was $70.5 \pm 54.5 \text{ ng g}^{-1} \text{ DW}$ in 2015, which was close to the amount obtained in 2014 ($70.9 \pm 1.7 \text{ ng g}^{-1} \text{ DW}$). In BT, extracted DNA was more abundant than in TT and yielded $\sim 120.1 \pm 25.2$ in 2014 and $135.7 \pm 25.9 \text{ ng g}^{-1} \text{ DW}$ in 2015 (Fig. 4.9; Table 4.1). In contrast, DNA content in the underlying soil layers (S1, S2 and S3) was significantly higher as evidenced by strong visible bands (Figs. 4.8b, 4.8c, C.2). S1

had the highest amount of extracted DNA with $3363.8 \pm 760.2 \text{ ng g}^{-1}$ of DW soil in 2015, which represented a 41% increase compared to 2014. For the deeper layers of the profile (T, S2 and S3), their DNA contents declined from 2014 to 2015: (i) T layer from 1323.2 ± 176.6 to $719.0 \pm 163.2 \text{ ng g}^{-1}$ (a 46% drop) (ii) S2 layer from 2402.3 ± 141.1 to $1531.0 \pm 92.3 \text{ ng g}^{-1}$ (a 36% decrease) (iii) S3 layer from 2483.7 ± 327.9 to $2021.0 \pm 236.4 \text{ ng g}^{-1}$ (a 20% decrease). Based on the obtained results, the concentrations of DNA varied in the buried by tephra layers from one year to another, but this variation in all layers was not statistically significant ($p = 0.2104$).

FUNGAL DIVERSITY The molecular assessment of tephra and soil fungal communities was performed via PCR amplification of extracted DNA using fungal-specific PCR primers (Fig. 4.10) and separation of the different PCR amplicons using DGGE (Fig. 4.11a).

Table 4.1: Chemical and microbiological properties of Mt. Etna volcanic deposits and buried soils collected in 2014 and 2015.

ID	pH	TOM (%)	TOC (g kg ⁻¹)	TN (g kg ⁻¹)	C/N	WC (%)	DNA amount (ng g ⁻¹)	FB (μg g ⁻¹)	FB/TOC, (%)	Ribotype number
2014										
TT-14	6.6 ± 0.1a	0a	0.8 ± 0.2a	0.1 ± 0.0a	5.6 ± 1.1	0.2 ± 0.2a	70.9 ± 1.7a	134 ± 11a	17.0 ± 4.6	
BT-14	6.4 ± 0.1 b	0.5 b	3.6 ± 0.7b	0.2 ± 0.0b	17.8 ± 3.6	0.5 ± 0.8a	120.1 ± 25.2b	363 ± 205b	10.8 ± 9.1	
S1-14	5.6 ± 0.0d	23.1 d	115.8 ± 11.7d	3.4 ± 0.4d	34.0 ± 0.7	2.2 ± 0.4b	1890.6 ± 415.1d	9775 ± 2980d	8.0 ± 2.2	
T-14	6.1 ± 0.1 c	0.9c	5.1 ± 1.2c	0.3 ± 0.0c	20.0 ± 1.8	0.3 ± 0.4a	1323.2 ± 176.6c	1278 ± 404c	24.3 ± 6.6	
S2-14	5.7 ± 0.1e	12.3e	58.6 ± 5.5e	1.9 ± 0.2e	30.8 ± 1.0	2.5 ± 0.6b	2402.3 ± 141.1e	4452 ± 1124e	7.2 ± 1.8	
S3-14	5.5	7.2	30.3 ± 4.4	1.1 ± 0.1	27.0 ± 1.7		2483.7 ± 327.9			
2015										
TT-15	6.5 ± 0.1	0	1.1 ± 0.4	0.2 ± 0.0	7.1 ± 2.6	0.0 ± 0.0	70.5 ± 54.5	151.0 ± 49.0	15.1 ± 6.3	2.3 ± 2.6
BT-15	6.4 ± 0.1	0.5	3.0 ± 1.3	0.2 ± 0.0	17.1 ± 5.5	1.7 ± 3.1	135.7 ± 25.9	565.0 ± 344.0	18.8 ± 9.7	10.0 ± 4.2
S1-15	5.2 ± 0.1	21.8	107.6 ± 10.4	3.2 ± 0.2	33.5 ± 1.8	24.1 ± 5.2	3363.8 ± 760.2	5346 ± 572	4.8 ± 0.9	9.3 ± 2.2
T-15	5.9 ± 0.1	0.9	5.3 ± 0.4	0.3 ± 0.0	20.2 ± 1.4	1.8 ± 0.9	719.0 ± 163.2	663 ± 151	12.0 ± 2.9	10.0 ± 3.9
S2-15	5.7 ± 0.1	13.1	60.0 ± 11.6	1.8 ± 0.4	32.8 ± 2.0	8.7 ± 2.9	1531.0 ± 92.3	2548 ± 750	4.3 ± 1.9	8.5 ± 2.9
S3-15	5.7 ± 0.1	8.4	42.2 ± 3.8	1.3 ± 0.2	33.5 ± 2.2	13.4 ± 1.5	2021.0 ± 236.4	1186 ± 244	2.7 ± 0.8	7.5 ± 3.5
A- B		A-A	A-A	A-A			A-A	A-A		

The values for pH, TOM, TOC, TN, C/N, WC, DNA amount (2015), FB and ribotype number represent the mean ± SD of four repetitions and DNA amount (2014) - two repetitions. Different capital letters indicate significant differences between values in 2014 and 2015 for each presented tephra and soil property (BLS). Different small letters indicate significant differences between mean values obtained for TT, BT, T, S1 and S2 in both years (Pairwise comparisons using Wilcoxon rank sum test, $p < 0.05$).

PCR cycling conditions were optimized for the selected ITS region and enabled the detection of fungi-specific PCR amplicons in tephra and soil samples for both years. By comparing with the DNA ladder, the size of all amplicons was estimated as ~ 700 bp (Fig. 4.10). The separation of PCR amplicons by DGGE allowed us to obtain a fingerprint of the fungal community characterized by a distinct DNA profile containing different PCR amplicons with different GC% contents. Identification of fungal species was achieved by purification and sequencing of specific bands collected in the DGGE profile of the tephra and soil samples. This approach, performed for samples collected in 2014 (Table 4.2), revealed the presence of *Ceratobasidium sp.* (Basidiomycota) and *Lophium sp.* (Ascomycota) in the TT and BT, respectively. The presence of *Pseudotomentella sp.* (Basidiomycota) was confirmed in S1 and T layers. *Umbelopsis sp.* (Zygomycota), *Mortierella sp.* (Zygomycota) and *Russula sp.* (Basidiomycota) were detected in S2. *Russula sp.* (Basidiomycota) was also found to be present in S3 together with *Trichloma sp.* (Basidiomycota). The well-defined bands of *Pseudotomentella sp.* (Basidiomycota) and *Russula sp.* (Basidiomycota) were found throughout the whole volcanic soil profile (including the BT), at the exception of TT, for which smearing of the bands complicated the definition of the presence of these species.

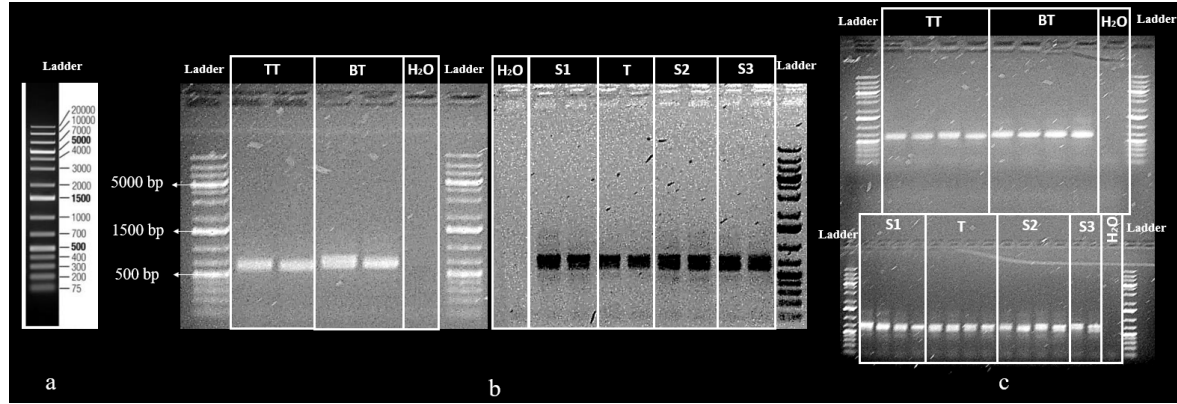


Figure 4.10: O'GeneRuler 1 kb Plus **DNA** Ladder representing the known quantities of nucleic acids (0.1 μg per μL **DNA** ladder) (a). This calibration **DNA** Ladder bands varies from 75 to 20000 bp. Note that the same ladder is present on the right and left-end side of each gel. According to this ladder, the estimated size of the amplicons is 700 bp. **PCR-DGGE** products of the samples collected in 2014 and 2015, respectively, amplified with ITS1 Myers/ITS4-KY01 primers for the ITS1-5.8SrRNA-ITS2 region present in all members of fungal community (b and c). Polymerization temperature is 47 °C. Lanes correspond to the following field replicates: TT, BT, S1, T, S2 and S3; H₂O – blank.

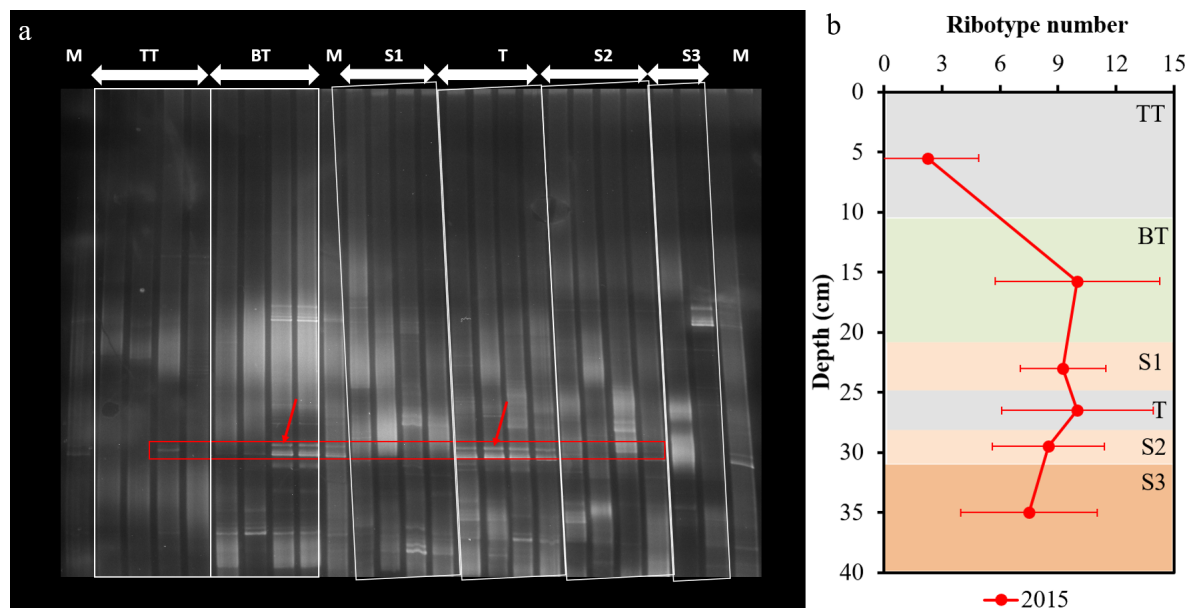


Figure 4.11: DGGE profiles of fungal amplicons of the same size, but different sequences, representing communities in tephra and soils in 2015 along the volcanic soil profile (TT, BT, S1, T, S2 and S3) (a). White rectangles on the gel lanes indicate position of individual bands, i.e. ribotypes. Numbers of DGGE ribotypes in tephra and soil collected in 2015 (b). Mean $\pm$ SD of four field replicates are shown.

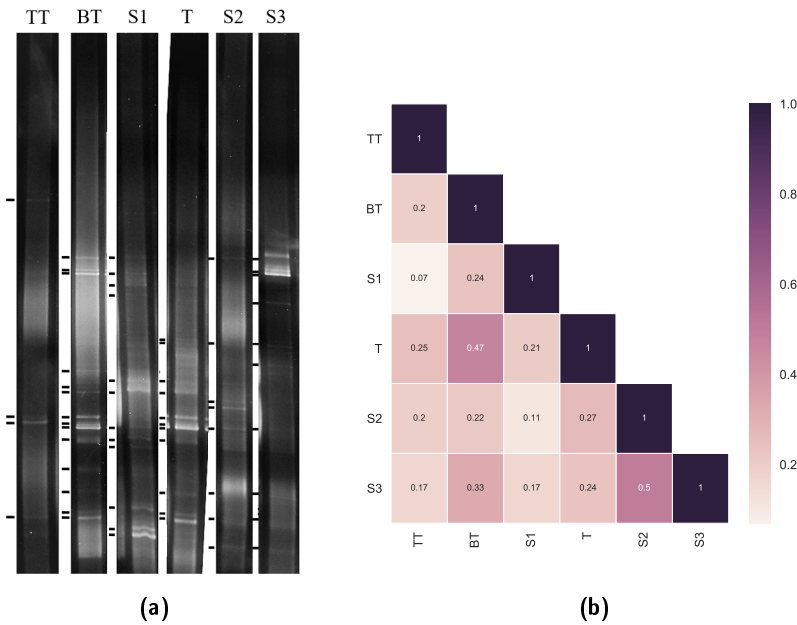


Figure 4.12: **DGGE** lanes of tephra and soil replicates from 2015 (a). Heatmap based on Jaccard estimations of fungal ribotype similarity in tephra and soil collected in 2015 as measured from **DGGE** bands (1.0 corresponds to the ribotype complete similarity)(b).

The fingerprinting of fungal community in the tephra and soil samples collected in 2015 was performed via the separation of **PCR** amplicons by **DGGE** (Fig. 4.11a). Considering that each separated band contains a unique and specific sequence of a ribotype (or taxon, or phylotype), the number of bands is taken as a proxy of taxonomic richness (i.e. number of taxa) within the sample. The **DNA** patterns for TT samples were typically characterized by much lower diversity than that of the deeper layers, as evidenced by the very low number of bands (2.3 ± 2.6 bands) (Fig. 4.11b). Two out of four replicates even showed no **DGGE** bands (lanes TT in Fig. 4.11a). Average number of bands in the deeper layers of the profile stayed rather constant up to

the bottom of the profile (lanes BT-S3 in Fig. 4.11a) and fall in the same range of values : 10.0 ± 4.2 , 9.3 ± 2.2 , 10.0 ± 3.9 , 8.5 ± 2.9 and 7.5 ± 3.5 bands for BT, S1, T, S2 and S3, respectively (Fig. 4.11b).

Jaccard analysis on replicates with the most clear band separation was used to assess the presence of identical ribotypes in different tephra and soil pairs of samples collected in 2015. The results indicated substantial differences in ribotypes between fungal communities in almost all studied tephra and soil layers (Figs. 4.12a, 4.12b). The TT fungal communities were the least similar with underlying S1 soil layer, with Jaccard index of 0.07. The best similarity (0.47-0.50) was found between BT and T, and between S2 and S3 communities. Higher Jaccard index (0.33) compared to others was also found for BT and S2. The BT and T layers were characterized by the presence of particularly intense two bands with the same electrophoretic behavior (Fig. 4.11a, indicated by red arrows), possibly because of the abundant population.

4.4 DISCUSSION

4.4.1 *Evidence for fast fungal colonization of freshly-deposited tephra*

We conducted a two-year study to characterize the pioneering communities of microorganisms colonizing freshly-deposited tephra from Mt. Etna eruption (on November 23, 2013) and underlying soil over a 2-year period. Fungi were particularly targeted as their symbiotic associations with tree -i.e. mycorrhiza- have been shown experimentally to actively colonize and alter fresh volcanic rocks (i.e. basalt) within a few month periods ([74]). Our present study in coniferous forest subjected to the deposition of fresh tephra tends to support this earlier finding.

Within only 10 months after deposition, fungal colonization of tephra was extensive in the lower portion of tephra layer (BT) as evidenced by

Table 4.2: Fungal species in tephra and soil, identified by [DGGE](#).

Tephra and soil layers	Fungi species
TT	<i>Ceratobasidium sp. (Bas.)</i>
BT	<i>Lophium sp. (Asc.)</i>
S1	<i>Pseudotomentella sp. (Bas.)</i>
T	<i>Pseudotomentella sp.(Bas.)</i>
S2	<i>Umbelopsis sp. (Zyg.)</i>
	<i>Mortierella sp.(Zyg.)</i>
	<i>Russula sp. (Bas.)</i>
S3	<i>Trichloma sp. (Bas.)</i>
	<i>Russula sp. (Bas.)</i>

the development of dense fungal networks that entangle whole tephra particles (Figs. 4.6a, c, d). Fungal biomass in BT layer is indeed significant (300 to $500 \mu\text{g g}^{-1}$) and increases slightly over time between 2014 and 2015, although fungi occurrence seems highly heterogeneous as evidenced by the large variability of the ergosterol content (Fig. 4.7, Table 4.1). Despite fewer filaments being observed in TT compared to BT by fluorescence-based technique (Fig. 4.6b), the ergosterol extraction confirms the presence of fungi in TT after 10 months of tephra deposition (Fig. 4.7). However, FB in TT is low (100 - $150 \mu\text{g g}^{-1}$) and relatively stable between 2014 and 2015, although temporal trend for TT is difficult to assess due to high variability of a ergosterol content in the 2015 samples. Detailed SEM inspection of TT particle surface failed to detect fungal mycelium, suggesting, that the fungi began its colonization by 'exploring' the interior of the tephra particles. The ergosterol results are further confirmed by the fact that PCR products could be obtained from TT and BT samples using the fungi-specific primer pair Myers ITS1 and ITS4-KY01 (Fig. 4.10) (despite the low amount of DNA extracted in both samples - Figs. 4.8 and 4.9). This successful PCR allowed for further sample manipulation in order to obtain the fungal ribotypes and species in distinct layers along the volcanic soil profile (discussed in detail in Section 4.4.3).

Overall, the first results suggest that fungal colonization of freshly-deposited tephra (TT+BT) was well underway 10 months after tephra deposition. Although well investigated in monoculture, nutrient-rich media, the determination of fungal growth rate is rarely determined in natural soil conditions. [19] derived an average hyphal growth rate of $\sim 10 \mu\text{m day}^{-1}$ over biotite surface in axenic mycorrhizal experiments while some studies suggest hyphal turnover rates varying from 7 to 145 days. This would mean that a hypothetical single hypha, surviving 10 months would grow to ~ 3 cm. Therefore, it is possible that hypha was able to colonize the BT layer from S1 layer.

Despite the absence of herbaceous vegetation at our sampling site within the two first years after tephra deposition, the **TOC** was significantly higher in BT compared to TT for both 2014 and 2015 samplings (Table 4.1). The **TOC** content in TT ($0.8 \pm 0.2 \text{ g kg}^{-1}$ in 2014; $1.1 \pm 0.4 \text{ g kg}^{-1}$ in 2015) was comparable to that in ~ 2.5 -3 years-old deposit from Puyehue-Cord on Caulle Volcanic Complex eruption in natural forest ($1.3 \pm 0.3 \text{ g kg}^{-1}$ after ~ 2.5 year, $1.9 \pm 0.9 \text{ g kg}^{-1}$ after ~ 3 years); in 9-11 years-old Miyake-jima deposit (0.2 - 0.7 g kg^{-1}) and in a 31 years-old Hawaiian scoria ($> 2 \text{ mm}$) deposit ($2 \pm 1 \text{ g kg}^{-1}$) ([31]; [50]; [42]). In contrast, **TOC** levels in BT were 4-18 times higher and comparable to those found in a 41 years-old Hawaiian scoria ($> 2 \text{ mm}$) deposit ($5 \pm 1 \text{ g kg}^{-1}$) ([50]). Our results show that **TOC** in freshly-deposited tephra is partly originating from 'living' (at least, recently) **FB**, which accounts for an estimated 10 to 19% of **TOC** (Table 4.1). The rest may come from atmospheric deposition ([82]), litter fall from the nearby trees, lateral transport or run-off of OM (particularly for TT), potentially, bacterial biomass, fungal exudation or extra polymeric substances (EPS). In deglaciated soils, the contribution of microorganisms (cyanobacteria) to carbon and nitrogen accumulation has previously been suggested ([75]). [31] suggested that, after tephra deposition, surviving canopy and understory provided seeds and organic matter to the tephra layer, creating more favourable conditions for plants, invertebrates and microorganisms that would induce faster soil development. In our study, there seems to be a stronger contribution of fungi to tephra **TOC** than to the buried soil **TOC** (Table 4.1), which suggests the important role of fungi in **TOC** accumulation in both soils and freshly-deposited tephra.

This reasoning is further strengthened by the positive correlation in our profile between **TOM**, **TOC**, **TN**, **WC** and **FB**, both for 2014 and 2015 (e.g., for 2015 $r^2 = 0.86, 0.87, 0.87$ and 0.78 , respectively). Our observation of the presence of **FB** in tephra layers in detectable quantities differs drastically from the study of [42], for which concentration of ergosterol was below the detection limit, implying a minor (if any) fungal contribution to the overall microbial community in their vol-

canic deposit and buried soils. According to those authors, it would take over 43 years to accumulate $\sim 1 \text{ g kg}^{-1}$ of **TOC** in their volcanic deposit. This is in strong contrast with the trend we observe in our site where ~ 0.8 to 1 and 3 g kg^{-1} of **TOC** (for TT and BT, respectively) were accumulated in less than a year.

Our study further reveals an early establishment of fungi communities in newly exposed tephra (< 1 year). This is especially true for the BT layer, which is in direct contact with buried soil and where **FB** is significant ($565 \pm 344 \text{ } \mu\text{g g}^{-1}$ **DW**) and increasing over time despite lower contents of water, organic matter and nitrogen compared to the buried soil layer (S1). The colonization of a fresh mineral surface by fungi, particularly ectomycorrhizal fungi, has previously been reported as an important process for biological weathering and biogeochemical cycling of the elements ([80]). The study of recent Hawaiian volcanic deposits (41 - 300 years) showed evidence of colonization by microorganisms despite the substrate acidity, inability to retain water and the absence of vegetation, organic matter and the lack of fixed nitrogen ([41]). All these tephra characteristics are the main environmental stresses affecting bacteria propagation (e.g., [69]; [32]). Fungi tend to have higher tolerance to the environmental stressors ([12]; [69]) being able to grow and survive in harsh conditions ([60]) as supported by our study.

4.4.2 *Variability of fungal distribution over the volcanic soil profile*

Compared to S1, S2 and S3, tephra layers (TT, BT and T) contained ~ 11 , 11 , 9 and 6 times less **TOC**, **TOM**, **FB** and **DNA** content, respectively in 2014 and 2015 (Table 4.1). Based on these results, our volcanic forest soils are environments with very distinct and sharp vertical stratification most likely due to the intermittent deposition of volcanic material, which alter or terminate the soil formation processes. In the latter case, tephra can become the parent material for a new soil ([78], Chapter 72).

In our experiment, the decrease in TOM with depth in various buried soils from S1 to S3 (Fig. 4.5e, Table 4.1) could result from a more recent litter input in S1 in comparison to S2 and S3, in which fresh input is probably limited, and where decomposition would have reduced the TOM content ([89]).

Interestingly, this drop in TOM content from S1 to S3 is mirrored by a concomitant decrease in FB (Fig. 4.7, Table 4.1). The declining FB from S1 to S3 can partly be explained by differences in organic matter quality and abundance ([89]; [59]). Except for mycorrhizal association, which benefits from C-photosynthates from their host-plant, microbiota, including saprophytic fungi, are often limited in soils by labile carbon (easily available organics) ([43]; [73]). As such, the absence of fresh litter input in the S2 and S3 soil layers due to the multiple tephra covers may decrease the organic matter pools on which fungi thrive ([91]).

Lower values of the FB in tephra layers (TT, BT and T) comparing to soil (S1, S2 and S3) FB could be partly attributed to the low amount of organic matter in the former materials ([89]) and by the limited exposure time (at least for BT and TT). As saprotrophic and some ectomycorrhizal fungi (*Russula*) with saprotrophic abilities obtain their energy by degrading organic matter ([89] and references therein), they would preferentially inhabit OM-rich substrate, such as soil layers (S1, S2 and S3) in the profile. However, the fact that tephra colonization by microorganisms was induced after 10 months of tephra deposition may partly be attributed to the higher TOM availability (i. e. higher content of labile TOM) in tephra than in soil layers. In our experiment, the hypothesis of difference in TOM quality between tephra and soil layers may be supported by lower tephra C/N ratio relative to soil C/N ratio, which is commonly used as an index TOC availability for microorganisms (Fig. 4.5f)([49]; [43]; [42]). Our assumption is also supported by the study of [31], who suggested that the higher enzymatic activity (referred to organic matter) in tephra as compared to forest floor is

attributed to the fact that forest floor is richer in recalcitrant organic matter.

For a more detailed exploration of the fungal community composition in tephra and soil layers, we applied more sensitive methods relying on nucleic acid extraction and DGGE technique that can detect community members with a relative abundance higher than 1 % of the total community ([62]). In all of our samples, the PCR-DGGE products had very consistent size of ~ 700 bp (Fig. 4.10). This is very much in line with the in silico PCR amplification using the primer pair ITS1 and ITS4-KY01 of the genome of *Russula brevipes* (Accession number: FJ845429), which gives an amplicon size of 699 bp (Fig. C.3). Taking into account the 40-mer GC clamp, this gives us a PCR amplicon size of 739 bp. In itself, this result suggests only small genetic variation within the species present in our soils profile ([67]).

DGGE analysis of PCR amplicons (Fig. 4.11) of each tephra and each soil layer provided us with semi-quantitative information on fungal diversity. The mean ribotype number showed that composition of the soil fungal communities was similar between the layers except for TT that had a much lower ribotype number (Fig. 4.11b). The relative stability of fungal communities between different horizons was previously reported in forest soils ([2]). Jaccard analysis of DGGE data confirmed the specificity of TT in terms of fungal communities in comparison to the buried soil layer (S1) of the profile (Figs. 4.12a and 4.12b).

A very reduced number of the fungal ribotypes in TT (compared to the underlying tephra and soil layers) may be due to environmental conditions and substrate specificity. Besides TOC content, a substrate pH usually may affect the abundance of fungal community ([90]). However, the pH in both TT and BT layers was near-neutral and most likely did not have a significant effect of fungi activity. The seasonal variations of temperature and WC are probably not a major parameter that can affect the fungal community composition, as sampling was

performed in September in both years. This suggests that in this case, environmental conditions do not seem to have major control on the number of fungal ribotypes in TT relative to underlying soil layers.

4.4.3 Identification and origin of fungi in the freshly-deposited tephra

The question of fungal origin on tephra was raised in a previous study by [22]. These authors hypothesised that the primary inoculum of the early and subsequent colonizers could be coming from the underlying soil and from the mixing soil and plant debris with tephra. A number of fungi species were identified from the [DGGE](#) analysis carried out in the buried soil and tephra layers sampled in 2014. We found *Umbelopsis* sp. (Zygomycota), *Mortierella* sp. (Zygomycota), *Russula* sp. (Basidiomycota), *Tricholoma* sp. (Basidiomycota), *Pseudotomentella* sp. (Basidiomycota), *Ceratobasidium* sp. (Basidiomycota) and *Lophium* sp. (Ascomycota).

This above list involves several ectomycorrhizal fungi, such as *Russula* sp., *Pseudotomentella* sp. and *Tricholoma* sp. commonly found in symbiosis with conifer trees. *Russula*, which was found in both tephra and soil layers, is one of the most abundant and common in conifer forests in Europe and North America, suggesting that this species is a good competitor and plays a key functioning role in [ECM](#) communities ([45]). *Russula* can associate symbiotically with a wide range of plant/tree host ([84]) and particularly with *Pinus nigra* ([85]), the main tree species of our study area. [68] identified *Russula* sp. in association with *Pinus montezumae* on volcanic soils at Sierra del Chichinautzin, Mexico. In temperate to boreal regions, it has been suggested that fungal communities evolve from *ascomycetes* being prevalent in surface litter to dominance of *basidiomycetous* fungi in the deeper soil horizons with [ECM](#) *Russulales* being the most abundant genus ([89]). The increase in the relative proportion of [ECM](#) taxa (and thus *Basidiomycota*) with soil depth was also reported in other studies ([56]; [30]; [25]). Our results, indicating the presence of *Russula* genus across the entire soil profile

(down to 40 cm depth), are thus in accordance with previous works. At our site, one may speculate that *Russula sp.* had formed ectomycorrhizal association with *Pinus nigra* before tephra deposition (in S1), survived the disturbances related to the eruption, and at a later stage started to colonize the tephra layer (BT) directly in contact with S1 ([22]; [39]).

In addition to *Russula sp.*, another ectomycorrhizal fungi - *Pseudotomentella sp.* - was identified from our DGGE analysis in S1 and T layers. This genus can also form ectomycorrhizal associations, in particular, with *Pinus sp.* ([85]) and is commonly found in Europe and North America soils. It belongs to the *Thelephoraceae* family, which was shown to grow in conifer forests ([85]). Thus, the occurrence of this fungi species at our sampling site located in conifer forest on Mt. Etna is in line with previous studies. The colonization of soil and tephra layers by *Pseudotomentella sp.* could have been a result of vertical upward fungi distribution from deeper layers in the volcanic soil profile.

In the deepest soil layer (S3) of the soil profile, *Tricholoma sp.* was identified. This genus - a member of *Tricholomataceae* family - can also form ectomycorrhizal symbiosis with different species of coniferous and broad-leaved trees ([57]).

Umbelopsis sp. and *Mortierella sp.* are zygomycetous fungi that were identified solely in the S2 layer. Both fungal species can form endophytic association in the xylem of healthy and declining conifers, particularly in *Picea mariana* in boreal forest ([81]). However *Umbelopsis sp.* also have saprotrophic capabilities and are found in forest leaf litter, while some species, such as *U. nana*, are proxy of mineral-soil in forest ([81]).

Mortierella sp. is a common and ubiquitous fungal genus ([86]) with species, such as *Mortierella verticilla sp.* found in moist but well-drained forest soils (similar to our study site). *Mortierella elongata* grows well

in a range of soil types having pH of 4 to 7, and shows preference for litter and upper organic soil horizons. The S2 layer at our study site seems favourable for the growth of these fungi.

Ceratobasidium sp. was found in TT and *Lophium* sp. was identified only in BT layers. *Lophium* is a genus part of the *Mytilinidiaceae* family, which are wood-decomposers, especially on gymnosperms ([76]). *Ceratobasidium* sp. is a ubiquitous genus of saprotrophic fungi that produces fruiting bodies on dead stems and plant detritus. The occurrence of *Ceratobasidium* sp. and *Lophium* sp. in the TT and BT tephra layers may be related to the presence of *Pinus nigra* wood and needles mixed with tephra particle during deposition or at later stage via aerial deposition.

The presence of species capable of ectomycorrhizal association with the surrounding tree species lead us to believe that this type of symbiotic associations can play a major role in the colonization of freshly deposited tephra. Ectomycorrhizal fungi are, indeed, less affected by the minute OC content of tephra substrate as they rely on the C-photosynthate supply from their host-plants for their metabolic needs ([80]). Also, freshly-deposited tephra contains slightly higher P concentrations (potentially also more bioavailable) than the buried soils and tephra, which might be a cause of the extensive colonization by ectomycorrhizal symbiotic fungi ([79]; [74]). In 2015, the P content of TT and BT layers was 2407 ± 36 and 2283 ± 88 mg kg⁻¹, respectively, while the P content in S1 layer was 1923 ± 136 mg kg⁻¹ (detailed results are not presented). Therefore, it is possible in our soil profile that the colonization of BT layers was pioneered by ectomycorrhizal fungi from underlying soil S1. In this respect, it is remarkable to find in BT and in the underlying soil layers *Pseudotomentella* sp. and *Russula* sp., which are both able of ectomycorrhizal associations with *Pinus* sp., the genus of trees that inhabits our site. The similarities of ribotypes identified by DGGE (in 2015) between the BT and T, S2 and S3, and finally between BT and S2 samples may also support the hypothesis that

colonization of BT was most likely accomplished from the underlying buried soils. Comparing to TT, the higher **FB** in the layer BT in contact with buried soil (S1), where **FB** was the largest ($> 9000 \mu\text{g g}^{-1}$ in 2014), also suggests that fungi invasion of tephra was driven by an upward growth of fungal networks from the underlying S1 soil layer. However, other sources of fungi in TT, for instance from the air microbiome, cannot be excluded. This might be true for *Ceratobasidium sp.* and *Lophium sp.* identified solely in TT and BT, which might originate from aerial dissemination from nearby areas. If one considers that the colonization is mainly driven by underlying fungal community, another aspect (besides **TOC** and **pH**) that can explain the limited number of taxons/ribotypes in TT is the relatively large thickness of tephra deposit (i. e. 20 cm TT+ BT), which renders TT less accessible to fungi growing from S1 (compared to BT).

4.4.4 *Tephra as potential ecological niche for fungi*

Amongst other processes, such as priority effect (historical contingency and order of arrival) and environmental selection, niche partitioning has been defined as major determinant of the fungal community structure ([66]). In our study, we consider that the tephra deposit represents a new favourable niche for certain fungi types present in the tephra as pioneers as evidenced by the fast colonization by fungi over a 10-month period after eruption. Although no strong evidence of mineral weathering could be detected so far (at least using macroscopic proxies such as **TRB** - Fig. 4.5a), this extensive colonization of BT will have a great impact on vegetation succession and soil formation as discussed in [64] and [37]. The growth in BT of vast fungal mycelium was most likely initially due to the hyphae ability from S1 to detect and exploit nutrient-rich patches in tephra, independent of the location of the root tips ([11]; [80]). Despite the fact that tephra is very porous material that drains water easily and, therefore, may represent a drier and less favourable substrate for microorganism growth compared to soil, fungi

tend to have an edge to colonize freshly-deposited tephra as they are distinguished by aerial growth and they are more tolerant to dry conditions than bacteria ([12]).

The question of community assembly and its evolution over time is hotly debated ([37]; [23]). The effect of the order and timing of the past events on community assembly (in other words, historical contingency) is of a great importance. Historical contingency can be biotic (i.e. colonization by new species during community assembly) and abiotic (i.e. disturbances, such as earthquakes or volcanic eruptions). Several factors affect the temporal community heterogeneity, such as climate change, nutrient pulses etc., while spatial heterogeneity is defined by stochastic (priority effects) and abiotic (legacy effects) factors. Priority effects imply the interaction and influence of species on each other depending on the order in which they colonize a given site ([37]; [23]). Legacy effects imply the influence of the residual environment on community assembly in newly established environment ([23]). Priority effects take place solely when the population of pioneering groups grows quickly so that those groups pre-empt or change ecological niches considerably before the onset of late-arriving species ([37]). Otherwise, community structure becomes deterministic and each species occupies the most-suited ecological niche ([37]). Therefore, deterministic effects imply the niche-based species selection, induced by competition and niche differentiation ([23]). The species are more competitive and more functionally different in high-resource environments, while they are more stress-tolerant and more similar in functioning at low-nutrient niche dimensionality (low diversity in resources) ([23]). The tephra deposition on forest soil after the volcanic eruption of Mt. Etna on November 23, 2013, resulted in a strong disturbance of this forest setting but also in the formation of a new environment with fresh tephra becoming a new, free niche available for community assembly. In this context, our study contributes to the knowledge in biotic and abiotic historical contingency. The legacy effects play a pivotal role as the underlying soil, especially S1, being a residual habitat, most likely

drives community assembly processes in the tephra layer in BT at this stage and most likely in TT in the coming years.

4.5 CONCLUSIONS

In recent decades, molecular techniques have allowed us to gain a much better insight into fungal assembly and succession in soils ([66]). However, numerous ecosystems are poorly understood, in particular areas exposed to deposition of volcanic material. In this study, we characterized the pioneering communities of microorganisms (with a focus on fungi) that colonize freshly-deposited tephra from Mt. Etna eruption on November 23, 2013, over a 2-year period.

We observed a fast development (<10 months) of fungal communities in freshly-deposited tephra. This colonization was more evident in the fresh tephra layer in direct contact with the buried soil and we speculate that ectomycorrhizal fungi were spearheading this expansion. These symbiotic fungi are non saprophytic (i. e. they are less dependant on the OM content of the substrate on which they grow) and they are also able to detect nutrient-rich patches in the fresh, un-weathered tephra. Results of molecular analyses tend to support this idea as we identified at least two ectomycorrhizal fungi species (*Russula sp.* and *Pseudotomentella sp.*) able to associate with the surrounding *Pinus sp.* Overall, our results show that freshly deposited tephra represents a potential ecological niche for fungi. Over time, fungi will contribute to the TOC accumulation, in freshly-deposited tephra, and as such they will be important agent of soil formation and thus of the re-vegetation process.

Further monitoring of this area would provide a unique opportunity to study the microbial community assembly over time with a known history of the site disturbance. This knowledge will improve our understanding of how communities evolve and interact between each other

in response to natural disturbance and global changes in the future environment.

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EFFECTS OF A VOLCANIC ASH DEPOSIT ON THE GROWTH AND LEAF PHYSIOLOGY OF TOMATO PLANTS (*SOLANUM LYCOPERSICUM*)

5.1 ABSTRACT

Background and aims. Explosive volcanic eruptions are the most powerful and destructive, with ash (i.e. tephra particle size <2 mm) being sometimes deposited in agricultural areas. Ash on plants can lead to irreversible damage, cause severe crop losses and therefore serious economic consequences. However, the mechanisms of the effects of ash on plants have been poorly investigated. Here, we present a study on the impact of ash deposit on plant growth, photosynthesis and antioxidant status.

Methods. Tomato plants (*Solanum lycopersicum*, cv. Aisla Craig) were cultivated in nutrient solution under controlled environmental conditions and received ash deposition according to a one-event scenario (S1, 90 g/plant) or a multiple event scenario (S2; 3 applications of 30 g/plant) using ash from the 1991 Mt. Pinatubo eruption. The experiment lasted two weeks and involved six replicates.

Results. Both scenarios induced an increase in shoot length and a decrease in shoot and root dry weights. Net photosynthesis was reduced by up to 20% while leaf temperature increased from 1 to 12%. Actual PS2 efficiency and photochemical quenching decreased while non-photochemical quenching increased at the beginning of the S1 and S2 treatments. S1 reduced sugar concentration during the first week of treatment, while S2 - during the entire experimental period.

Conclusions. Ash affected plants by reducing the amount of light captured by the leaf, thereby reducing sugar synthesis and reducing plant growth. Moreover, we suggest that carboxylation efficiency may be reduced in ash-treated plants in relation to an alteration of the Calvin cycle or other components of the dark phase of photosynthesis. Plants exposed to a one-event scenario were able to acclimate more efficiently than those exposed to a multiple ash deposit.

5.2 INTRODUCTION

Ash is the most widespread product of explosive volcanic eruptions ([48]). Ash is a pyroclastic material less than 2 mm in size generated by fragmentation of an erupting magma. It is comprised mostly of silicate glass and minerals in variable proportions, although hydrothermally-altered rock fragments may also be incorporated. Volcanic ash is a low specific surface area material (i.e. few $\text{m}^2 \text{g}^{-1}$) and its shape varies greatly depending on several eruption-related parameters ([13];[18]). Fine ash ($<63 \mu\text{m}$) can be transported high in the atmosphere by the wind for very long distances before deposition. Significant ash deposits can be found hundreds to thousands of kilometres from the volcanic vent ([50]). An ash deposit can also be re-suspended in the atmosphere long after it was formed, generating a secondary hazard ([54], [4]).

Deposition of volcanic ash on terrestrial ecosystems can cause various physical and chemical damages to plants (see review [5]). Current knowledge on ash damage to plants largely emanate from studies carried out in the aftermath of the May 18, 1980 Plinian eruption of Mt. St. Helens, USA. These revealed plant smothering and burial, breaking of stems and leaves and altered pollination ([9]; [12]; [10]). Leaves that had survived mechanical damages showed increased necrotic lesions and chlorotic spots ([31]), possibly in response to dissolution of sulphate and chloride salts adsorbed on the ash particles ([9]). Moreover,

[44] and [19] reported decreased growth and reduced photosynthetic activity in ash-affected coniferous trees. The presence of ash particles on foliage may hamper photosynthetic activity directly due to leaf abscission and mechanical damage, but also indirectly by lowering the quantity of radiation reaching the leaves ([9]; [19]).

The majority of active volcanoes are located in developing regions where the agricultural sector accounts for a substantial part of national economies ([30]). This is not a coincidence as soils developed on ash deposits are typically fertile ([46]; [11]). As such, they support more than 10% of the world's population although they cover only around 1% of the surface of the Earth. However, agriculture in these active volcanic regions is vulnerable to ash emissions. For example, the agricultural sector experienced more damage than any other industry after the 2006 eruption of Merapi volcano, Indonesia, mostly due to ash fall on crops ([56]). Similarly, the reduction in alfalfa and apple production caused by the ash from Mt St. Helens in 1980 was equivalent to losses of $\approx$ \$ 35 and \$ 15 million, respectively ([9]).

Clearly, there should be a strong incentive for describing how an ash deposit on the foliage of plants (and more specifically crop plants) affects plant physiology and growth stages of development. However, surprisingly, studies that provide a detailed understanding of the types, intensity, duration and mechanisms of impacts generated by ash deposition on plants are not available. This contrasts with the works that investigate the interaction of industrial and road dust with plant foliage (e.g., [42]). Currently, the degree of damage to plants affected by volcanic ash is tentatively related to thickness of the deposit ([44]; [19]; [3]). This is a crude and oversimplistic approach that calls for more detailed investigations. Such new knowledge can contribute to guide mitigation plans in order to minimise crop losses and to aid capacity-building for sustainable agriculture and rural development in active volcanic regions ([10]; [52]).

Here we present the results of an experimental study in which tomato plants were exposed to simulated volcanic ash deposition under controlled environmental conditions. Tomato is one of the most important vegetable crops in the world and it is cultivated in many active volcanic regions (e.g., [56]; [54]; [55]). Two ash deposition scenarios were simulated: a single and a multiple event. Each experiment lasted two weeks and we monitored plant growth and photosynthesis activity as well as various biochemical parameters related to water regulation and oxidative stress in the tomato plants.

5.3 MATERIALS AND METHODS

5.3.1 *Plant material and growing conditions*

Seeds of *Solanum lycopersicum* (cv. Ailsa Craig) were purchased from Tomato Genetic Resource Center (TRGC; University of California, Davis, USA). The seeds were germinated in a peat compost substrate (80% bark, 20% pumice and Scotts Osmocote ®(Scotts Company LLC, Marysville, OH, USA) at 25 °C. Two weeks after germination, tomato seedlings were transplanted to 1.5-L plastic pot (two seedlings per pot) filled up to 1.3 L with a hydroponic solution (pH 5.8) prepared according to [21] containing (in mM): 5 KNO₃, 5.5 Ca(NO₃)₂, 1 NH₄H₂PO₄, 0.5 MgSO₄, and (in μM) 25 KCl, 10 H₃BO₄, 1 MnSO₄, 0.25 CuSO₄, 1 ZnSO₄, 10 (NH₄)₆Mo₇O and 1.87 g L⁻¹ Fe-EDTA. Solutions were renewed weekly. The tomato plants were anchored to perforated polystyrene plates (11.5 x 16 cm) floating on top of the solution. The experiment was carried out in a phytotron room under a 16 h photoperiod. Light intensity was maintained between 210 to 250 μmol m⁻² s⁻¹ using fluorescent lamps (Master TL-D reflex Super 80 58W/840, Philips, Brussels, Belgium). The day and night temperatures were maintained at 25 ± 2 and 22 ± 1.5 °C, respectively. Relative humidity was 65-75%.

5.3.2 Ash deposition scenarios

Ash from the 1991 eruption of Mt. Pinatubo volcano, Philippines, was used in all experiments (Fig. 5.1). The ash has a dacitic composition (≈ 63 wt. SiO_2). It was collected in March 2016 from a Plinian air fall deposit ≈ 6 km north-east from the crater. The material was sieved through a 250- μm nylon mesh prior to application to tomato plants. A subsample was analysed for particle size distribution by laser diffraction (COULTER LS 100Q). A suspension consisting of distilled water (approximately 200–250 ml) and ash (<0.5 g) was first subjected to sonication for 30 s and then passed through the analyser. The Fraunhofer diffraction equation ([38]) was applied to obtain the volumetric percentage of particles of different sizes. Fifty percent of the ash particles (D_{50}) were smaller than 48 μm (Fig. D.1).

Ash deposition on tomato plants was simulated after one week of seedling adaptation to the hydroponic solution. A 135 cm-high PVC tube comprised of four sections separated by three meshes (1-mm opening) was placed vertically over each pot (Fig. 5.2). Ash was introduced through a 250 μm mesh resting upon the top of the tube. The deposition of ash was facilitated by gently shaking and rotating the top mesh and tapping the PVC tube. Paper strips were fixed around the pot to protect the hydroponic solution from ash contamination. The procedure allowed formation of an ash deposit uniformly distributed across the adaxial surfaces of the tomato leaves.

Two scenarios of ash deposition were considered (Fig. 5.3): (i) a one-event scenario (S1) in which 90 g of ash were applied once (Fig. 5.3a), and (ii) a multiple-event scenario (S2) in which 30 g of ash were applied three times (i.e. on days 0, 3 and 8 after transplanting seedlings to hydroponic solutions) to the tomato plants (Fig. 5.3b). In S1, the ash load was ≈ 0.2 g per cm^2 of leaf surface area (deposit thickness of ≈ 0.12 cm). In S2, each ash application corresponded to ≈ 0.1 g per cm^2 leaf surface area (deposit thickness of ≈ 0.06 cm). The exper-

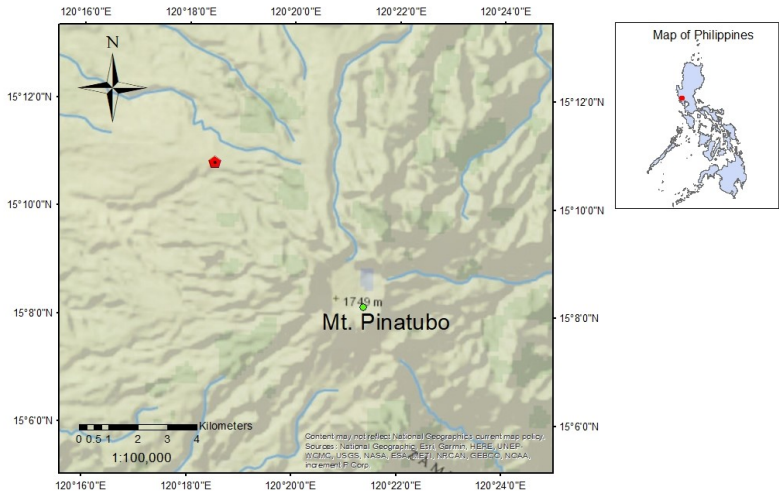


Figure 5.1: Map of sampling site. The sampling site is shown with red dot.

iments lasted two weeks and involved six replicates (i.e. total of 48 ash-affected plants). The control consisted of 48 plants that did not receive ash.

5.3.3 *Leaf temperature, photosynthesis- and plant growth-related parameters*

Leaf temperature (T), photosynthesis- and plant growth-related parameters were monitored eight times throughout the experiment on days 1, 2, 3, 4, 7, 9, 11 and 14 after the first ash application. As shown in Figure 5.3, the stem bore four leaves at the time of the first ash application. Physiological measurements were performed on leaf “n° 4”

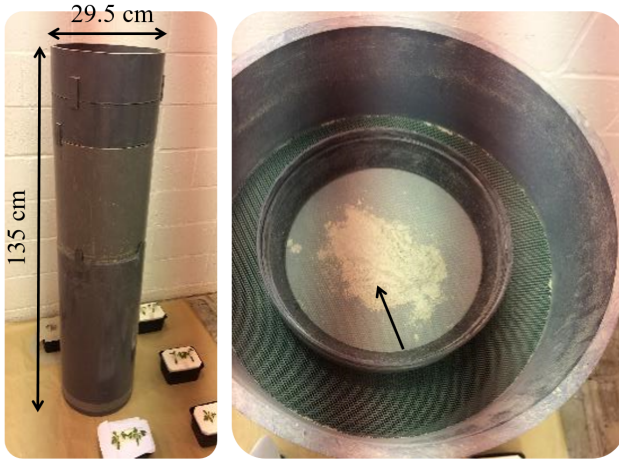


Figure 5.2: Photographs of the 135 cm-high polyvinyl chloride (PVC) tube used for ash application to the plants (left); ash (indicated by black arrow) applied to the plants through a 250 μm mesh resting upon the top of the tube (right).

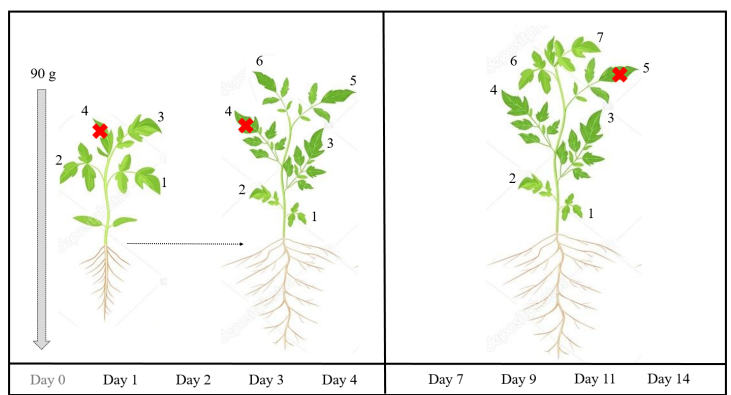
(numbered acropetally) on days 1, 2, 3 and 4. Subsequent physiological measurements were performed on leaf “n° 5”. Thus, leaf “n° 5” was never exposed to ash in the S1 scenario, whereas it received ash on days 3 and 8 in the S2 scenario.

Leaf temperature

The temperature of the control and ash-treated leaves was estimated using an infrared camera (FLIR-ix7). For each treatment (including control), the leaf temperature (T), was determined on six plants.

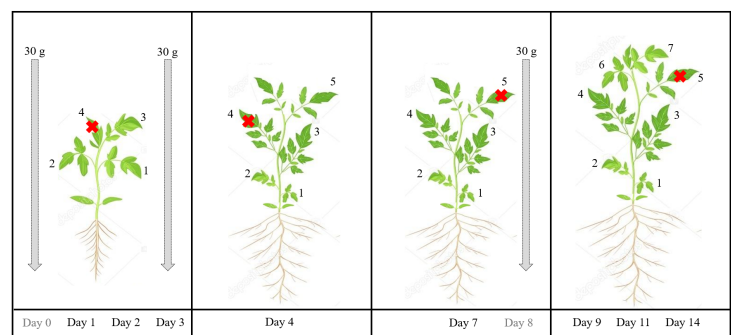
Photosynthesis-related parameters

Leaf *Stomatal conductance* (g_s) was measured for each treatment (including control) on six plants with an AP4 diffusion porometer (DeltaTDevices Ltd., Cambridge, UK). Instantaneous CO_2 assimilation (*Net*



✖ - Leaf used for measurements of physiological parameters

(a) S1 scenario



✖ - Leaf used for measurements of physiological parameters

(b) S2 scenario

Figure 5.3: Schematics of the scenarios of ash application corresponding to a single event (a, S1) or to a multiple event (b, S2). In S1, plants received 90 g of ash on, day 0, while in scenario 2, plants received 30 g of ash at days 0, 3 and 8. Leaf temperature, physiological and plant-growth-related measurements were performed 8 times during a two-week treatment. The position of the leaf used for leaf temperature, physiological and plant-growth-related measurements is indicated with a red cross and corresponds to a leaf n° 4 (numbered acropetally) for day 1 until day 4, and to a leaf n° 5 for day 7 until day 14. Hence, leaf n° 5 appeared after the ash treatment in scenario 1, while it received two ash applications in S2. The images of tomatoes were taken from www.nl.depositphotos.com/111421292.

photosynthesis (A) under ambient conditions (i.e. 400 ppmv CO_2), *Inter-cellular* CO_2 concentration (c_i) and *Instantaneous transpiration* (E) were obtained using an infrared gas analyser (LCA4 8.7 ADC, Bioscience, Hertfordshire, UK; [53]). Gas exchange was measured with a Parkinson leaf cuvette for 1 min (20 measurements min^{-1}) under an air flow of 300 mL min^{-1} . All measurements were performed between 9 am and 12 pm. *Water use efficiency* (WUE) was calculated as the ratio of the rate of CO_2 assimilation (net photosynthesis) to the rate of transpiration.

Chlorophyll fluorescence analysis of the leaves was carried out with a portable pulse-modulated chlorophyll fluorimeter (FMS2; Hansatech, King's Lynn, UK). The leaflet's middle sections were dark acclimated for 20 min before the measurement. Minimal fluorescence yield, F_0 , was obtained by recording the modulated light ($0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$). The maximal fluorescence level (F_m) with all photosystem II (PS2) reaction centers open was determined using a 0.8 s saturating pulse at $8500 \mu\text{mol m}^{-2} \text{s}^{-1}$ in dark-adapted leaves. The leaves were then continuously illuminated with white actinic light ($320 \mu\text{mol m}^{-2} \text{s}^{-1}$) during 3 min; the steady-state value of fluorescence (F_s) was recorded. A second saturating pulse at $8500 \mu\text{mol m}^{-2} \text{s}^{-1}$ was imposed to determine maximal fluorescence level in the light-adapted state (F_m'). The actinic light was removed and the minimal fluorescence level in the light-adapted state (F_0') was determined by illuminating the leaf with a 3-s pulse of far-red light. *The maximal efficiency of PS2's photochemistry in the dark-adapted state* (F_v/F_m) = $(F_m - F_0)/F_m$, *Photochemical quenching coefficient* (qP) = $(F_m' - F_s)/(F_m' - F_0')$, *Non-photochemical quenching* (NPQ) = $(F_m - F_m')/F_m'$ and *actual PS2 efficiency* (Φ_{PS2} = $(F_m' - F_s)/F_m'$) were calculated according to [32]. The results of the chlorophyll fluorescence measurements are presented for days 4 and 14.

Plant growth-related parameters

The control and ash-treated plants from S1 and S2 were harvested each time after measuring the physiological parameters, i.e. on days 1 – 4 and 7, 9, 11 and 14. All harvested plants were washed with demineralized water and carefully wiped. The *Shoot height* (H) and *Root length* (L) were obtained for the six replicates. The shoot and root parts were separated and weighed, and then incubated in an oven at 76 °C during 48 h for determining the *Shoot dry weight* (DW_s) and *Root dry weight* (DW_r) and *Shoot water content* (WC_s) and *Root water content* (WC_r). The three plants remaining in each treatment were kept for biochemical analyses (see below). Freshly separated shoots and roots were ground and homogenized in a mortar and in the presence of liquid nitrogen before being stored in a freezer at - 80 °C until required.

5.3.4 *Biochemical parameters*

For each ash application scenario, a set of biochemical analyses was conducted on two composite samples of tomato shoots (the whole aerial part, except the stems) and roots collected on days 1, 2, 3 and 4, and days 7, 9, 11 and 14, respectively. All analyses were performed in triplicates.

Chlorophyll and carotenoid contents

Total chlorophyll (($chl\ a + chl\ b$)) and *Carotenoid* (C_{car}) concentrations were determined using 100 mg of fresh leaf material ground with 10 mL of acetone 80% (v/v) in a pre-chilled mortar. The absorbance of the extract was recorded at 663.2, 646.8, and 470 nm using a Shimadzu UV-1800 spectrophotometer (Kyoto-Japan). Pigments concentrations were calculated according to [29].

Sugar and protein contents

Approximately 0.4 g of fresh shoot and root materials were ground to a fine powder in liquid nitrogen. The ground material was extracted three times with 4 ml of 70% ethanol (w/v) and centrifuged for 15 min at 6600 g at 4 °C. For total soluble sugar quantification, 200 µl of the supernatant were reacted with 1 ml of anthrone solution (0.5 g anthrone, 250 ml H₂SO₄ 95% and 12.5 ml distilled water) during 10 min at 100 °C ([57]). The absorbance was read at 625 nm and a standard curve was established using glucose.

Total soluble proteins were extracted from 50 mg of fresh ground shoots and roots weighed in a 1 mL Eppendorf. Five hundred µL of a 100 mM phosphate buffer (pH 7.4) were added to the Eppendorf prior to vortexing and centrifuging the samples for 15 min at 12000 g and -4 °C. Protein concentrations were determined according to [6] using 1.25 mL of Biorad protein reagent added to 25 µL of supernatant aliquot. The absorbance was measured after vortexing at 595 nm.

Lipid peroxidation and protein-bound carbonyl levels

The level of lipid peroxidation in the control and ash-treated plants was assessed from the concentration of *Malondialdehyde* (MDA) as determined by the thiobarbituric acid (TBA) reaction ([17]). Ground fresh shoots and roots (0.25g) were homogenized in 5 mL of 5 % (w/v) trichloroacetic acid (TCA) containing 1.25 % glycerol. The homogenate was centrifuged at 12000 g for 10 min at 4 °C and filtered on Wathman No. 1 filter paper. Two ml of TBA (0.67%) were added to 2 mL of supernatant and the mixture was heated at 100 °C for 30 min. The reaction was stopped by placing the reaction tubes in an ice bath. The samples were centrifuged at 12000 g for 1 min and their absorbance was measured at 532 nm. The results were corrected by subtracting the non-specific absorbance component as measured at 600 nm. The concentration of MDA (nmol g⁻¹ DW) was calculated using an extinc-

tion coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

The reaction with 2,4-dinitrophenylhydrazine (DNPH) allowed carbonyl quantification in the shoots ([28]). Three replicates and their respective blanks were used for each determination. Samples were incubated with 0.2% (v/v) Triton-X and 1% (w/v) streptomycin sulphate for 20 min to remove nucleic acids. After centrifuging at 6000 g, supernatants (400 μL) were mixed with 600 μL of 10 mM DNPH in 2M HCl. The blank was incubated in 2M HCl. After 1 h incubation at room temperature in the dark, proteins were precipitated with 10% (w/v) TCA, kept in the ice for 10 min before washing the pellets three times with 600 μL of ethanol/ethylacetate (1/1). The pellets were dissolved in 6 M guanidine hydrochloride in 20 mM potassium phosphate buffer (pH 2.3) and incubated at 37 °C for 30 min. The absorbance was measured at 370 nm. Carbonyl content was calculated using a molar absorption coefficient for aliphatic hydrazones of $22000 \text{ m}^{-1} \text{ cm}^{-1}$.

Total antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay ([7]) was applied to determine the total antioxidant activity both in hydrophilic and lipophilic extracts of tomato shoots. In brief, 1 g of frozen crushed shoot material was extracted with 10 mL methanol, incubated at 4 °C during 12 h and centrifuged at 10000 g for 20 min to obtain the *Hydrophilic antioxidant activity* (HAA). The supernatant was stored at -20 °C until required. The organic residue was redissolved in 10 mL of dichloromethane and used for determination of *Lipophilic antioxidant activity* (LAA). The samples were kept at -4 °C during 12 h and centrifuged at 10000 g for 20 min and then stored at -20 °C. The LAA and HAA were measured in triplicates for each treatment. The antioxidant activity of the methanol and dichloromethane extracts was measured using the DPPH radical discoloration assay. A stock solution was prepared by dissolving 24 mg DPPH in 100 mL methanol. The working solution was obtained by mixing 100 mL stock solution with 450 mL methanol to obtain an

absorbance of 1.1 ± 0.02 units at 515 nm. Shoot extracts (75 μL) were diluted with methanol (75 μL) and allowed to react with 2850 μL of the DPPH solution for 24 h in the dark. The discolouration caused by reduction of the cation by antioxidants from the sample was measured at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water soluble vitamin E analogue, was used to prepare a standard curve between 0 and 800 μM and activity was reported as micromolar Trolox equivalent antioxidant activity ($\mu\text{M TE g}^{-1}$ fresh weight).

5.3.5 Statistical analysis

All statistical analyses were performed using the SPSS 25.0© software. Significant differences in values obtained for biochemical parameters between treatments on days 1 – 4 and 7, 9, 11 and 14 were assessed with a one-way analysis of variance (ANOVA1) in combination with Tukey's test ($p < 0.05$). Treatment effects on leaf temperature, physiological parameters and plant-growth related parameters were evaluated by two-way ANOVA with days of measurements (or plant age) and treatments (control, S1 and S2) as factors. Principal component analysis (PCA) was performed with the R© software in order to transform the set of measured parameters (i.e. A , c_i , E , g_s , T , WC_r , WC_s , WUE , DW_r , DW_s , H , L , F_v/F_m , ΦPS_2 , qP , NPQ) into fewer variables that could describe changes in plant physiological condition ([23]).

5.4 RESULTS

All measured parameters (plant-growth related, physiological and leaf temperature) differed significantly with time (plant age) and between treatments as estimated by two-way ANOVA (Table D.1).

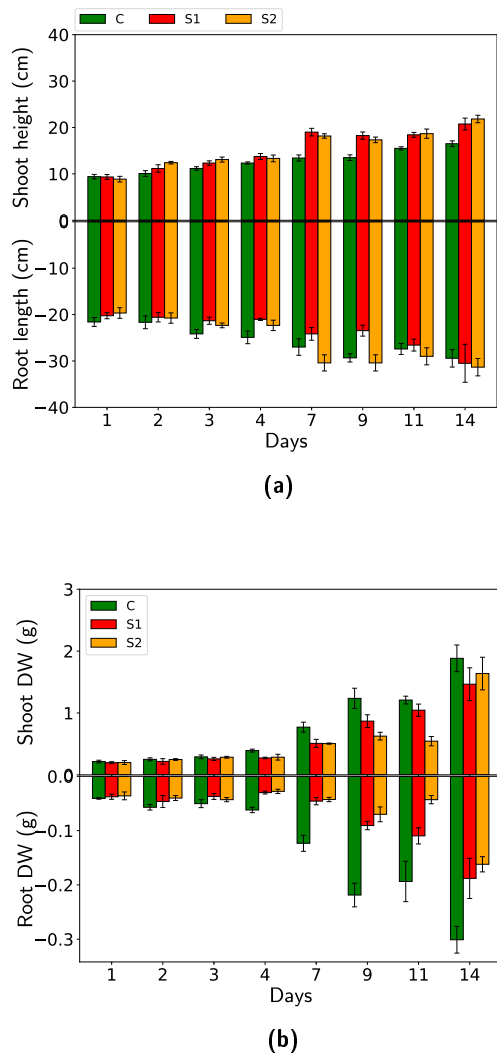


Figure 5.4: The height of the tomato shoots, measured from the interface of roots and shoots up to the highest leaf, and the length of the tomato roots. Results are the means of six sample replicates (a); biomass of dry tomato shoots and roots. Biomass is the mean of three sample replicates (b). Error bars represent SE from the mean.

5.4.1 *Plant growth*

Visual inspection of the ash-treated and control tomato leaves revealed that the former became lighter in colour within four days after ash application (Fig. D.2). The leaves were folded and wilted, and they were thinner and more brittle. After seven days, the ash-treated plants in both S1 and S2 were higher than the control plants (Fig. 5.4a). In contrast, no systematic increase in L was observed for the S1 and S2 plants. DW_s decreased in the ash-treated plants on day 4 and remained slightly lower than that of the control plants until the end of the experiment (Fig. 5.4b). The S2 plants exhibited lower DW_s values than those of the S1 plants on days 9 and 11. Ash application did not affect DW_r on the first three days, but drastically reduced it on the following days. The DW_r of the S1 and S2 plants in the second week of experiment were on average 48% and 62% lower, respectively, than the DW_r of the control plants (Fig. 5.4b). Ash deposition had only a marginal effect on leaf WC_s after the first four days of treatment in both S1 and S2 (Fig. 5.5). However, from day 7, leaf WC_s of the ash-treated plants was higher than that of the control plants. On days 9, 11 and 14, leaf WC_s was higher in the S2 plants compared with that in the S1 plants (Fig. 5.5). There was only slight difference for root WC_s between controls and ash-treated plants during the entire duration of the experiment.

5.4.2 *Photosynthesis-related parameters*

Table 5.1 shows the results of the photosynthesis-related parameters. Compared to the controls, ash treatment of the tomato plants did not alter the F_v/F_m values (measured on days 4 and 14). $\Phi PS2$ decreased on day 4 in both ash application scenarios, but it recovered to values similar to those found for the control plants at the end of the experiment. The presence of ash on leaves led to a reduction in qP on day 4, with the effect being more pronounced in S2. Similar to $\Phi PS2$, there was no difference in leaf qP values between the ash-treated and control

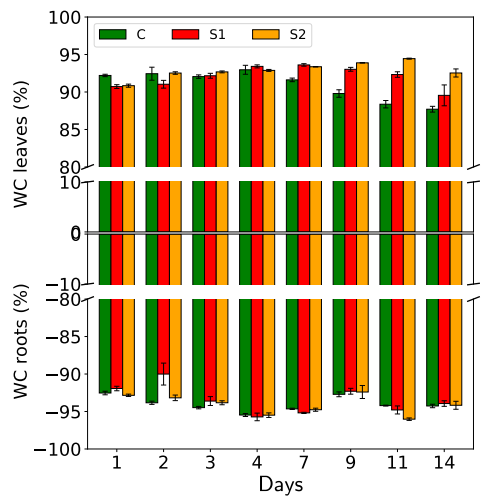


Figure 5.5: Water content in tomato shoots and roots. Water content is the mean of three sample replicates. Error bars represent SE from the mean.

plants measured on day 14. Non-photochemical quenching increased on day 4 in S1 and S2, but did not differ from the values measured for the control plants on day 14. The *(chl a + chl b)* and *Ccar* concentrations decreased on days 1 – 4 and remained low on days 7, 9, 11 and 14; the pigment concentrations were slightly lower in the S1 than in the S2 plants on days 1 – 4.

Ash on the surfaces of tomato leaves led to lower net photosynthesis (*A*) values (Fig. 5.6a), although no difference could be inferred between the two ash application scenarios. Intercellular CO₂ concentration (*c_i*) in the ash-treated plants was slightly higher than in the control plants on days 9 and 11 (data are not shown). In S1, *E* increased after day 1 in the ash-treated leaves as compared with the control plants. However, lower values were recorded at the end of the experiment (Fig. 5.6b). In S2, higher transpiration (*E*) values were found on days 1, 3, 7 and 9. Stomatal conductance values were lower in the S1 plants on days 1, 3

and 4, and in the S2 plants on days 1, 3, 4 and 7 as compared with the control measurements (Fig. 5.6c). In contrast, the S1 and S2 plants displayed slightly higher g_s values on days 9, 11 and 14. Instantaneous water use efficiency was significantly lower in the ash-treated plants than in the control plants, except on days 2 and 14 (Fig. 5.6d).

Finally, for both ash application scenarios, an increase in leaf temperature was recorded in the ash-treated plants on days 1, 2 and 9 (Figs. 5.7 and D.3). In S2, a higher temperature was also measured on day 14. Overall, the difference in leaf temperatures between the ash-treated and control leaves ranged between 1 and 12%.

Table 5.1: Photosynthesis-related parameters measured on days 4 and 14 after the first ash application. The results are the mean $\pm$ SE of six sample replicates. Chlorophyll and carotenoid contents are the mean $\pm$ SE of three sample replicates from days 1 – 4 and days 7, 9, 11 and 14.

	day 4			day 14		
	C	S1	S2	C	S1	S2
F_v/F_m	0.91 $\pm$ 0.00	0.91 $\pm$ 0.00	0.92 $\pm$ 0.00	0.91 $\pm$ 0.00	0.91 $\pm$ 0.00	0.92 $\pm$ 0.00
$\Phi PS2$	0.81 $\pm$ 0.01	0.68 $\pm$ 0.03	0.54 $\pm$ 0.07	0.87 $\pm$ 0.00	0.87 $\pm$ 0.01	0.86 $\pm$ 0.01
qP	0.92 $\pm$ 0.01	0.80 $\pm$ 0.04	0.62 $\pm$ 0.07	0.96 $\pm$ 0.00	0.98 $\pm$ 0.01	0.95 $\pm$ 0.01
NPQ	0.39 $\pm$ 0.06	0.68 $\pm$ 0.07	0.56 $\pm$ 0.08	0.13 $\pm$ 0.01	0.13 $\pm$ 0.02	0.23 $\pm$ 0.05
$(chl\ a + chl\ b), (mg\ g\ FW^{-1})$	1.56 $\pm$ 0.16a	0.59 $\pm$ 0.02b	0.85 $\pm$ 0.13b	1.44 $\pm$ 0.04a	0.87 $\pm$ 0.09b	0.74 $\pm$ 0.02b
$Ccar, (mg\ g\ FW^{-1})$	0.33 $\pm$ 0.01a	0.12 $\pm$ 0.00b	0.18 $\pm$ 0.03b	0.33 $\pm$ 0.01a	0.20 $\pm$ 0.02b	0.18 $\pm$ 0.02b

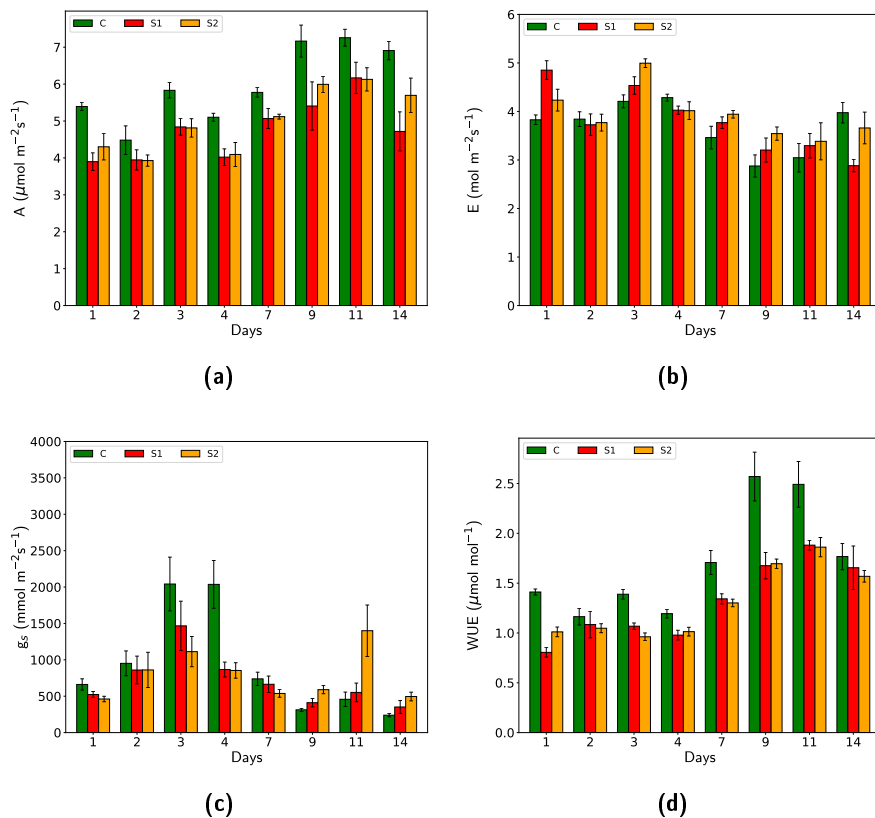


Figure 5.6: Net photosynthesis (a); instantaneous transpiration (b); stomatal conductance (c) and water use efficiency (d) of the controls, S1 and S2 leaves. Results are the means of six sample replicates. Error bars represent SE from the means.

5.4.3 Soluble sugars and protein contents

In both the ash-treated and control plants, the total soluble sugar concentrations in the shoot and root materials were systematically higher

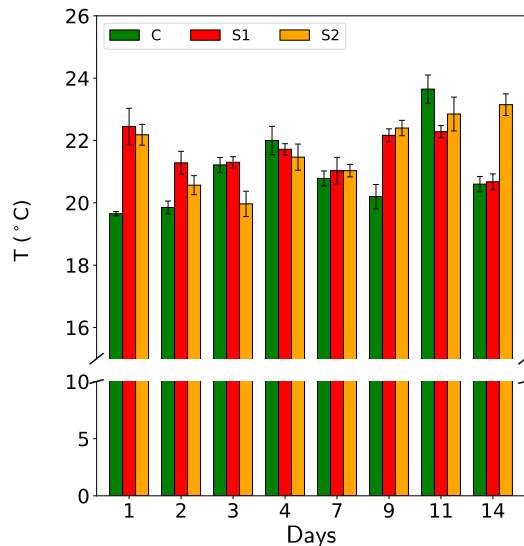


Figure 5.7: Temperature of the fully expanded leaves, obtained by IR-camera. Leaf temperature is the mean of six sample replicates. Error bars represent SE from the means.

on days 7, 9, 11 and 14 than on days 1 – 4. On days 1 – 4, the shoots of the ash-treated plants had much lower sugar concentrations (27 and 20% on average in S1 and S2, respectively) than the shoots of the control plants (Fig. 5.8a). The difference was only 3% at the end of the S1 experiment. In contrast, on days 7, 9, 11 and 14, the total sugar concentration was on average 41% lower in the S2 plant shoots than in the controls. Ash application in a single-event decreased the sugar content in roots by 27% (days 1 – 4) and 11% (days 7, 9, 11 and 14). On days 1 – 4, the root sugar content in the S2 plants was similar to that in the control plants, but it decreased significantly (~ 39% lower) at the end of the experiment (Fig. 5.8a).

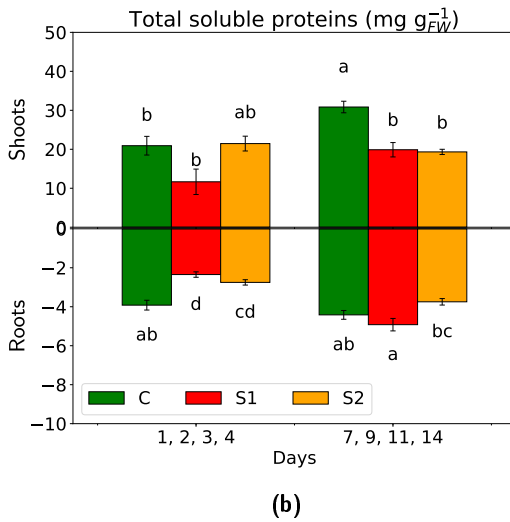
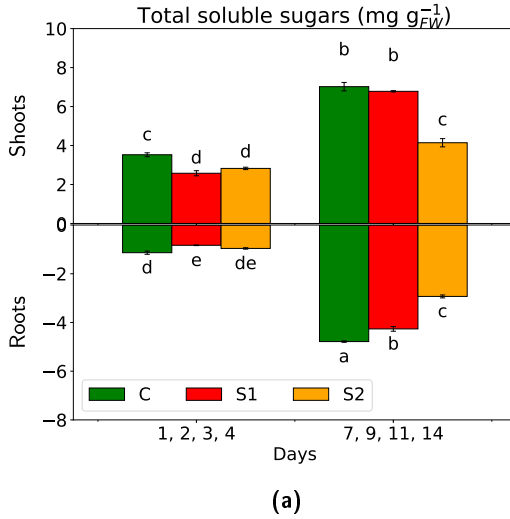


Figure 5.8: Total soluble sugar content (a) and total soluble protein content (b) in the controls, S1 and S2 tomato shoots and roots on days 1 – 4 and days 7, 9, 11 and 14. Results are the means of three sample replicates. Error bars represent SE from the means. Means with different letters are significantly different (Tukey's test, $p < 0.05$).

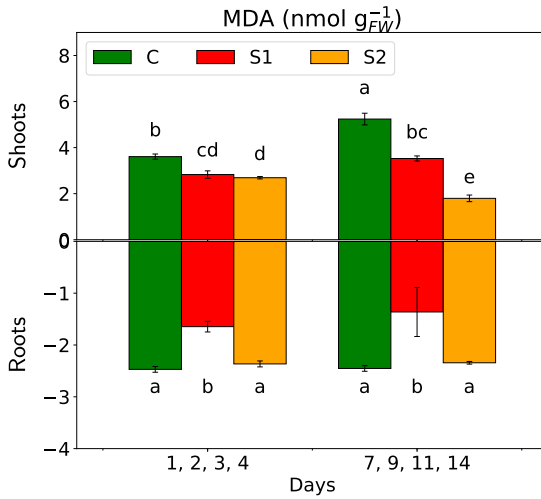
In S1, the protein concentration in shoots on days 1 – 4 decreased slightly in response to ash deposition, although this effect was not significant when compared to the control plants (Fig. 5.8b). The same observation applied to the S2 plants. In both ash application scenarios, the protein concentration in shoots on days 7, 9, 11 and 14 decreased by up to 37%. Root proteins in the S1 and S2 plants on days 1 – 4 were 40 and 30%, respectively, lower than in the control plants. On days 7, 9, 11 and 14, root protein concentrations in the S1 and S2 plants were not significantly different from those in the control samples.

5.4.4 *Oxidative stress-related parameters*

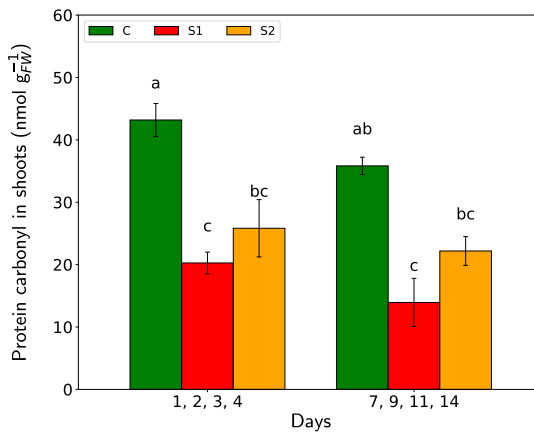
Shoot malondialdehyde (MDA) concentrations decreased significantly in both the S1 and S2 plants, and this effect was more pronounced at the end of the experiment (Fig. 5.9a). S1 and S2 plants on days 1 – 4 displayed shoot MDA concentration values 22 – 25% lower than those in the control plants. On days 7, 9, 11 and 14, the difference in MDA concentration between the ash-treated plants and controls was much larger in S2 (66%) than in S1 (33%). A significant reduction in root MDA concentration on days 1 – 4 and days 7, 9, 11 and 14 was observed only for the S1 plants (Fig. 5.9a).

Compared to the control plants, the concentrations of protein carbonyl groups in the shoots of the S1 and S2 plants were lower by 53% and 40% on days 1 – 4, respectively, and 61% and 38% on days 7, 9, 11 and 14, respectively, than those in the control plants (Fig. 5.9b). No significant difference was observed for the root parts of the ash-treated plants (data not shown).

Over the entire duration of the experiment, and regardless of the mode of ash application, the hydrophilic antioxidant activity (HAA) values in shoots of the treated plants were lower than those measured in the control plants (Fig. 5.10a). The differences on days 1 – 4 and days



(a)



(b)

Figure 5.9: MDA in the controls, S1 and S2 tomato shoots and roots on days 1 – 4 and days 7, 9, 11 and 14 (a); carbonyl contents in the controls, S1 and S2 tomato shoots on days 1 – 4 and days 7, 9, 11 and 14 (b). Results are the means of three sample replicates. Error bars represent SE from the means. Means with different letters are significantly different (Tukey's test, $p < 0.05$).

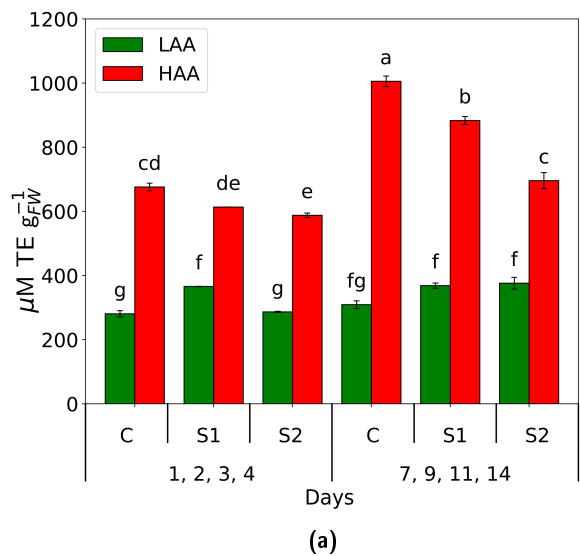


Figure 5.10: Hydrophilic (*HAA*) and lipophilic (*LAA*) antioxidant activity in tomato shoots on days 1 – 4 and days 7, 9, 11 and 14. Results are the means of three sample replicates. Error bars represent SE from the means. Means with different letters are significantly different (Tukey’s test, $p < 0.05$).

7, 9, 11 and 14 were 9% (S1) and 13% (S2), and 12% (S1) and 31% (S2), respectively. In contrast to the *HAA* measurements, lipophilic antioxidant activity (*LAA*) in the shoots of S1 plants was 23% higher than in the control shoots on days 1 – 4. No significant changes in *LAA* were observed between the shoots of S2 and those of the control plants. Similar *LAA* values were found on days 7, 9, 11 and 14 between the S1, S2 and control shoots.

5.5 DISCUSSION

5.5.1 *Effects of ash deposition on plant growth*

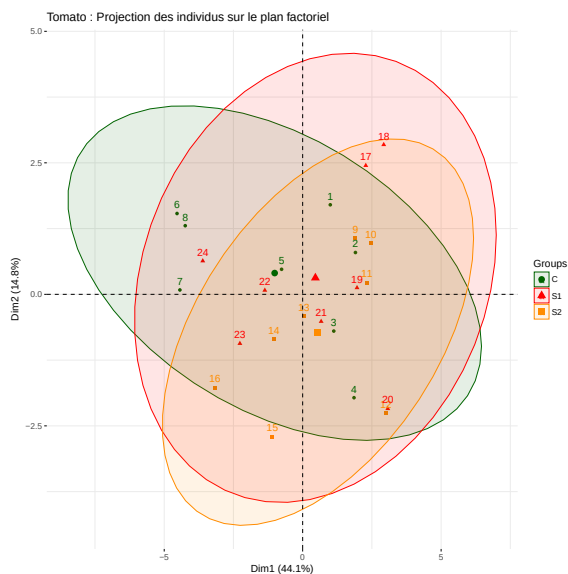
Most of the ash initially applied to tomato plants adhered to leaf surfaces during the two week-duration experiment. A small amount of ash (not quantified) was lost as the plant grew and leaves changed position. This underlines the important role of leaf position and characteristics in determining the ash residence time on vegetation ([5]). Tomatoes have pubescent leaves and in contrast to glabrous leaves, the presence of trichomes favours retention of solid particles such as ash ([9]; [31]; [56]) and dust of non-volcanic origin ([49]; [39]). Due to the weight of the ash deposit, leaf tilting occurred in response to a single ash deposition event (S1). The same phenomenon was also noticed in the multiple ash deposition scenario (S2), but it affected only the oldest leaves that had been subjected to more than one ash application. This observation is in accordance with previous field studies where the magnitude of plant damage correlated with the amount and frequency of ash deposition on foliage (e.g., [19]; [3]; [44]; [35]).

Along with the observed leaf damages, including folding, wilting (Figs. D.2 and D.3) and thinning, the decrease in DW_s measured in both ash application scenarios may be due to photosynthesis inhibition ([27]). Similarly, a significant decrease in plant growth parameters, accompanied by lower photosynthesis, has been suggested for plants exposed to cement (kiln) dust ([16] and references therein; [47]; [40]; [1]). After the 1980 eruption of Mt. St. Helens, [9]; [44]; [19] and [31] reported decreased growth, development of diseases, premature leaf abscission and fruit drop in plants affected by ash falls. [9] posited that the long-term presence of ash on leaves hampered photosynthesis and damaged the protective waxy cuticle of leaves, thereby lowering their resistance to stress. More recently, [56] hypothesised that leaf damages, such as shrivelling and wilting, on tomato plants exposed to ash emissions from the 2006 eruption of Mt. Merapi were due pri-

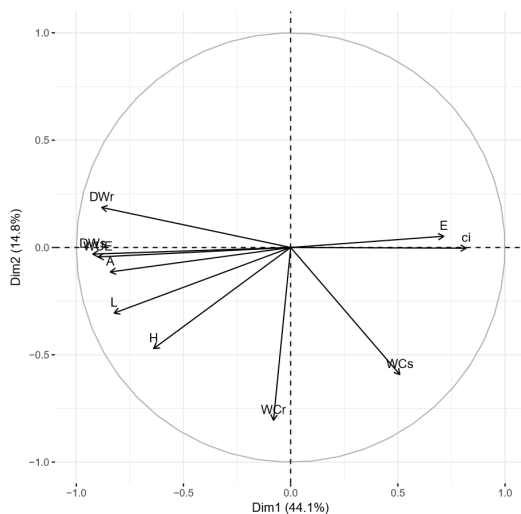
marily to the release of acidic ash leachates. However, this type of chemical interaction does not apply to our study as the tomato leaves were never in contact with liquid water. In addition, the ash material used in our study was exposed to rainwater prior to collection and therefore, the salts that may have been present on the ash surfaces had long been dissolved. Finally, despite a reduction in total biomass, the plants subjected to ash deposition showed a noticeable elongation of shoot height. This points to a change in plant morphology in response to ash application. Arguably, elongation of herb stems and rhizomes was also reported in areas affected by ash falls from Mt. St. Helens ([2], [3]). Shoot elongation associated with a decrease in dry weight may be considered as an etiolation process allowing the plant to overcome burial and the decrease in light interception resulting from the ash deposit.

5.5.2 *Effects of ash deposition on plant physiology and biochemical parameters*

Ash on tomato foliage induced various changes in 16 parameters (i.e. A , c_i , E , g_s , T , WC_r , WC_s , WUE , DW_r , DW_s , H , L , F_v/F_m , $\Phi PS2$, qP , NPQ). The PCA analysis was used for assessing plant growth and physiological condition of the differently-treated plants. Although the variants overlapped, the data points corresponding to the S1 and S2 ash-treated plants were slightly shifted with respect to the position of the control group (Fig. 5.11a). This suggests that different treatments can be distinguished between each other, despite similarities in the plant growth and physiological parameters. The vectors of the considered variables (plant growth and physiological parameters) presented in Figure 5.11b show that the first principal component (Dim1) is positively correlated with E and c_i . This suggests that these two parameters vary together. At the same time, the first principal component is negatively correlated with DW_s , DW_r , L , WUE , A , and to a lesser degree with H . Therefore, these parameters negatively correlate with E and c_i . The second



(a)



(b)

Figure 5.11: Principal Component Analysis of variability of parameters, tested for assessing growth and physiological condition of the controls and ash-treated plants (S1 and S2) (a); Vectors of the considered variables showing the correlation between them (b).

principal component (Dim2) shows that WC_s and WC_r are independent variables.

The chlorophyll fluorescence analyses (Table 5.1) reveal that the S1 and S2 plants underwent several, but short-lived, disorders in their photosynthetic machinery. On day 4, $\Phi PS2$ and qP decreased and NPQ increased in response to ash deposition, whereas F_v/F_m did not vary. Similarly, [37] did not observe a significant reduction of F_v/F_m for the *Avicennia marina* leaves covered by coal dust and suggested that no severe photoinhibition occurred. While the concentrations of photosynthetic pigments decreased, this can hardly explain the behaviour of the fluorescence-related parameters ($\Phi PS2$, qP and NPQ). Indeed, lower chlorophyll concentrations persisted on days 7, 9, 11 and 14, although chlorophyll fluorescence had fully recovered by the end of experiment (i.e. day 14). Following [51], this corroborates the idea that the relationship between chlorophyll content and chlorophyll fluorescence is not straightforward. Since the ash deposit did not alter F_v/F_m , the maximum quantum yield of $PS2$ was not affected and thus, QA-reducing $PS2$ reaction centers (where QA is a primary electron acceptor plastoquinone) may have remained connected to the light-harvesting complexes ([25]; [22]). However, lower qP values on day 4 suggest that the onset of carbon metabolism was delayed, or perhaps reduced, and that the proportion of open reaction centres decreased ([36]).

Non-photochemical quenching (NPQ) is a mechanism by which excess energy can be dissipated as heat ([36]). In our experiment, NPQ increased whereas qP decreased on day 4 (Table 5.1). Further, lower quantum efficiency of $PS2$ electron transport was measured, suggesting that the electron transport chain itself was altered. The decreased size of the light-harvesting antenna and $PS2$ inactivation may have influenced NPQ ([8]; [36]). Increased NPQ in the ash-treated plants may have been mediated by a particular group of carotenoid pigments of the xanthophyll cycle ([15]; [8]). However, the total carotenoid content in the ash-treated plants decreased (Table 5.1). Usually the xanthophyll

cycle is activated under light excess ([15]; [8]). In our experiment, the leaves were not exposed to a light excess but rather to a light deficit due to the presence of the ash deposit. While it is not possible to conclude about the mechanism responsible for the observed variations in NPQ on day 4 of the S1 and S2 ash treatments, the changes in F_v/F_m , $\Phi PS2$, qP and NPQ were transient, i.e. similar values of F_v/F_m , $\Phi PS2$, qP and NPQ for the ash-treated and control plants were recorded on day 14. Thus, at the photosystem level, repair and adaptation mechanisms operated efficiently, and in both ash deposition scenarios the leaves were able to perform the light-dependent phase of photosynthesis.

Net photosynthesis (A) inhibition was recorded throughout the entire experimental period (Fig. 5.6a). At Mt. St. Helens, almost complete suppression of photosynthesis was quoted for apple leaves covered with 1 mm of ash ([9]). In addition, particles of ash less than 2 μm were shown to clog leaf stomata, impeding gas exchange and altering leaf photosynthesis and temperature ([19]; [26]). Similarly, dust (industrial and road) deposition on foliage was shown to alter stomatal conductance due to blockage of leaf stomata ([43]; [45] and references therein; [16] and references therein; [20]). In our experiment, there was no clear evidence for photosynthesis inhibition due to partial closure of stomata by ash, although this effect may have occurred transiently on days 1, 3 and 4 (Fig. 5.6c). However, the ash deposit covered the adaxial face of the tomato leaves. Since the stomata in tomato plants occur mostly on the abaxial leaf surface ([33]), ash particles did not come in close proximity to the leaf guard cells. Another explanation of limited stomatal closure in the S1-treated plants is that the new leaves, appearing on the course of plant growth, were not exposed to ash treatment and, therefore, no stomata closure due to physical ash impact could have occurred.

A decrease in leaf A , likely explains the lower protein and sugar concentrations determined for the S1 and S2 plants (Fig. 5.8; [40]; [41]). Of note, root biomass (DW_r) was strongly reduced despite the fact that

the tomato plants were grown in a nutrient solution, which never received ash (Fig. 5.4b). The decrease in root sugar concentration upon ash treatment may account for reduced sugar translocation from the shoot to the root system (Fig. 5.8a). In natural conditions, a decrease in root growth would have a severe impact on nutrient and water absorption ([53]).

As shown in Figure 5.6b, an increase in transpiration (E) coincided with low stomatal conductance (g_s) values in the ash-treated foliage on days 1 and 3. However, there was no sign of water stress symptoms (Fig. 5.5). These apparently contradictory findings may relate to increased cuticular transpiration ([37]). Interestingly, the g_s values of leaves on the S2 plants were higher than those measured for the newly-developed leaves (not covered by ash) on the S1 plants on the last days of measurements. This result may support our assumption that guard cells of S2 plants were not clogged by ash particles and their function was not depressed. Moreover, this could suggest that S2-treated plants have adapted to the new conditions created by the ash deposit, for example, by changing the number of stomata, which could have helped to regulate the transpiration and to limit possible entry of particles into plant tissues ([40]). The decrease in net photosynthesis (A) combined with the increased E values resulted in a reduction of water use efficiency (WUE). While this ash-mediated effect was not a limiting factor for the tomato plants grown in hydroponic cultures, it may cause serious drought symptoms in plants under natural conditions ([58]).

Infrared thermography measurements have been used to monitor successfully leaf stomatal conductance (e.g., [24]; [59]). However, our data do not highlight a relationship between temperature and E or g_s in the ash-covered leaves on tomato plants (Figs. 5.6b, 5.6c, 5.7). [44] posited that light interception by the Mt. St. Helens ash deposit increased leaf temperature of fir trees. Ash on foliage also may affect photosynthetic activity indirectly by lowering the quantity of radiation reaching the leaf chloroplasts. For example, ash deposition on

pine foliage in excess of 10 g m^{-2} was shown to decrease the radiative flux captured by needles by up to 98% ([19]). The shading effect due to accumulation of cement dust on leaves was shown to reduce leaf chlorophyll concentration and photosynthesis (e.g., [47]; [42]). Thus, ash deposited on our tomato plants probably reduced the amount of light that could be captured by foliage, thereby causing a decrease in photosynthesis and an increase in leaf temperature. This would explain the leaf temperature increase measured on day 1 in both S1 and S2. However, in S1 scenario leaves were not exposed to the ash treatment on days 7, 9, 11 and 14, which implies that other mechanism than light interception by ash was responsible for photosynthesis reduction. The fact that intercellular CO_2 (c_i) increased suggests that carboxylation efficiency may be reduced in ash-treated plants in relation to an alteration of the Calvin cycle or other components of the dark phase of photosynthesis.

Malondialdehyde (*MDA*) and carbonyl residues are normally produced by oxidative damages of membrane lipids and proteins, respectively. In both S1 and S2, the tomato plants exposed to ash had lower concentrations of *MDA* and carbonyl residues than the control plants (Figs. 5.9a and 5.9b). This result is surprising as the generation of Reactive oxygen species (*ROS*) in plants subjected to environmental stress is widely documented (e.g., [14]). Our findings may reveal an efficient antioxidant defense mechanism in tomato plants exposed to ash. While the lipophilic antioxidants in the ash-covered leaves increased marginally in S1 (Fig. 5.10a), the concentrations of hydrophilic antioxidants decreased. Therefore, we rule out a role of cellular antioxidant defense. Alternatively, the shading effect of ash on foliage may be responsible for the subdued *MDA* and carbonyl residue concentrations. It is well known that chloroplasts are major sources of *ROS* ([34]; [14]). The amount of light that could reach chloroplasts in the ash-covered leaves may have been reduced to such extent that oxidative stress, and relatedly *MDA* and carbonyl residue production, was drastically hampered. This suggestion is compatible with a comparatively higher *ROS*

generation in the control tomato plants (Figs. 5.9a and 5.9b). Finally, the lack of evidence for oxidative stress in response to ash application is consistent with the view above that alteration of the fluorescence-related parameters at days 1 – 4 did not relate to structural disorders.

5.5.3 *Single versus multiple ash deposition*

The tomato plants responded differently to ash deposition in S1 and S2. Initially, (days 1 – 4), the single ash application had a greater impact on the plant biochemical parameters, such as root sugar, shoot and root protein (i.e. Figs. 5.8a and 5.8b). This may simply relate to the higher ash load received by the plant foliage in S1. Later, (days 7, 9, 11, 14), repeated ash deposition affected more the shoot and root sugar contents (Fig. 5.9a). The S2 plants on day 14 also displayed a clear increase in leaf temperature (Fig. 5.7). This difference in temperature noticed between the S1 and S2 plants may relate to the fact that the temperature and physiological measurements in the second week (days 7, 9, 11, 14) of the experiment were carried out on leaves “n° 5”. As already mentioned in section 2.3, leaves “n° 5” did not receive ash in S1, but they did in S2.

Nevertheless, the physiological parameters of leaves “n° 5” in S1 were also altered when compared to those of leaves in the same position on the control plants (Fig. 5.6). This would imply that ash deposited by a single event on tomato plants may have a knock-on effect on new organs. The fact that the root sugar and shoot protein contents of plants in S1 were lower as compared to these of controls on the first four days of experiment suggests that a single ash application was enough to affect the entire plant viability.

However, partial plant recovery had already taken place in S1 at the end of experiment as can be seen through the shoot sugar and root protein contents that were comparable to the control values. In

contrast, the stronger negative effect on these biochemical parameters observed in the multiple ash deposition scenario on days 7, 9, 11 and 14 highlights a cumulative and potentially more deleterious impact on plant functioning.

5.6 CONCLUSIONS

We simulated a single and a multiple event of ash deposition on tomato foliage in controlled environmental conditions and we monitored plant growth and various photosynthesis-related and biochemical parameters during two weeks. Our results suggest that the initial ash load on foliage dictates the severity of impacts on photosynthesis-related parameters, i.e. chlorophyll and sugar contents, and efficiency of the photosynthetic system. This effect was relatively short-lived as the plants were able to start recovering from ash-induced stress within two weeks. Nevertheless, a single ash deposition was sufficient to affect plant growth and thus, to reduce biomass, significantly. Multiple applications of smaller amounts of ash eventually led to stronger disturbances, more particularly in the second week of treatment. We argue that the quantity of radiation reaching the leaf chloroplasts of the ash-treated leaves was reduced through the shielding effect of the deposit. Moreover, we suggest that carboxylation efficiency may be reduced in ash-treated plants in relation to an alteration of the Calvin cycle or other components of the dark phase of photosynthesis. This probably explains the observed decreased in photosynthetic activity, which in turn led to lower sugar contents and reduction in plant growth. Unexpectedly, neither an oxidative damage nor an increase in antioxidant activity could be evidenced in the ash-treated plants. The reduced amount of light captured by the leaf chloroplasts may have strongly hampered development of an oxidative stress. Further studies will improve our understanding of ash effects on plants and their mechanisms, with particular emphasis on plant recovery.

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EFFECTS OF A VOLCANIC ASH DEPOSIT ON THE GROWTH AND LEAF PHYSIOLOGY OF RICE PLANTS (*POKKALI*)

6.1 ABSTRACT

During a volcanic explosive eruption, ash particles interact with terrestrial plants in various ways, often resulting in detrimental effects such as altered leaf physiology and reduced plant growth. In agricultural areas, ash deposition can lead to severe crop damages and significant economic losses. While many studies highlight the mechanical impact of ash fall on vegetation, little is known about the morphological, physiological and biochemical responses of plants to an ash deposit.

Here, we present the results of an experimental study aimed at better understanding of the physiological consequences of ash-leaf interaction. Ash from the 1991 Mt. Pinatubo eruption was applied to rice plants grown in controlled conditions in a phytotron. Two scenarios of ash deposition were considered: a one-event scenario (S1, 180 g/plant) and a multiple-event scenario (S2, 3 applications of 60 g/plant). The experiment lasted two weeks and involved six replicates.

Application of ash on rice plants resulted in leaf breakage, wilting and rolling. A leaf colour changed locally, on the folded and/or covered by ash leaf parts, and plant growth was reduced. The higher direct mechanical impact of S1 ash treatment on rice and delayed mechanical impact of S2 ash treatment were observed. We suggest that severity of the initial mechanical damage of rice is dictated by the amount of ash on the leaves, while the magnitude of mechanical impact over the longer time depends on the frequency of ash deposition.

In both ash deposition scenarios, the shoot biomass was reduced by up to ~ 50%. Net photosynthesis of the leaves covered by ash was always lower (by up to 32%) than that measured for the control plants. This decrease may be due to the lower quantity of radiation reaching the leaf chloroplasts of the ash-treated leaves due to the shielding effect of the deposit. Leaf temperature was higher in the affected plants than in the control plants by 1 - 17%. Our findings show the potential of rice to cope with unfavourable conditions by a single ash load (S1). In contrast, the S2 ash load leads to more detrimental effect on rice biochemical parameters (e. g., reduction of sugar and protein contents by 30%). The photosystem *PS2* was not altered by ash deposition. There was no oxidative damage neither, most probably due to the reduced amount of light captured by the leaf chloroplasts.

6.2 INTRODUCTION

Explosive volcanic eruptions are the most powerful and destructive, with ash (i.e. tephra with particle size less than 2 mm) clouds that can spread around the globe ([39], Part 4, Chapter 25). Notably, ash fall is the most frequent and far-reaching of volcanic hazards, and therefore one of the most threatening to society and surrounding environment ([20]; [7]). The ash emissions spaced over periods of varying length (from a few weeks to several years) may pose major problems to agricultural ([44]; ([45]; [10]) and natural ecosystems (e.g., [16]; [15]; [2], [3]; [4]; [13]). Ash falls and consequent ash redistribution over agricultural areas often lead to severe crop losses and therefore serious economic consequences. For instance, the ash deposits after eruption of Mt. St. Helens in 1980 diminished the production of crop, costing approximately \$ 100 million in 1980 or 7% of the normal crop value. The loss of alfalfa production was estimated as \$ 35 million, while the loss of apple production was about \$ 15 million ([9]). One of the more recent volcanic eruptions of Tungurahua in 2006 affected approximately 15 thousand farmers or 50 thousand hectares of cultivated ar-

eas. The eruption repeated in 2012 and 2014 and heavily damaged the agricultural sector ([6]). After the eruption of Mt. Hudson, southern Patagonia, Chile in 1991 the remobilised ash also severely damaged the agricultural economy of the region ([44]).

The vast majority of the knowledge on ash effects that may result in reduced crop production comes from field observations. However, the mechanisms of these effects have seldom been considered and represent an area requiring further study. Controlled experiments are important in order to understand the ash impact on vegetation and mechanisms driving these effects. Moreover, the use of plants that are widely cultured in areas that receive intermittent ash fall, will help to increase awareness of the potential risks for the crops. The present study was conducted to contribute to this knowledge by using the rice culture as a model plant.

The choice of the plant was based on the leaf characteristics, agricultural value in the volcanic areas and the experimental limitations (possibility to grow the chosen plant type under controlled conditions). Rice is widely cultivated in the areas in the close vicinity of the volcanoes (e.g., Philippines) and it is an important crop plant for the economic well-being of society. For instance, a volcanic material deposited after the Pinatubo eruption, Philippines in 1991 severely affected many areas relying on agriculture as a major industry. In those areas, rice was the principal crop food, and most rice fields were irrigated and planted to two rice crops per year. Almost 30000 ha or 18% of rice-lands in the major rice-producing provinces were affected by volcanic deposits ([34]). The main effects included the coverage of agricultural lands with particles and changes of soil properties ([23]). The poor rice harvest was reported both in terms of quantity and quality (e.g., reduction in the number of tillers, grains per panicle along with a decrease in grain yield by 20%) ([28]; [22]). The thickness of volcanic deposit may dictate the magnitude of its impact on rice. An ash layer of less than one mm would not cause damage, while five mm-thick deposit

may destroy the rice seed-heads ([46]). The 30 mm-thick deposit may reduce the number of grains, while the 90 mm-thick deposit may significantly decrease the total number of tillers ([22]). The rice maturity is also an important factor in determining the degree of physical damage. For example, rice would be vulnerable during mature stages, but resilient during initial stages of development ([46]).

These observations on the degree of damage to rice affected by volcanic ash, which are related to thickness of the deposit and rice maturity, are important, but oversimplistic. Further investigations are needed to describe how an ash deposit on the rice leaves affects plant physiology and growth stages of development. This new knowledge can contribute to guide mitigation plans in order to minimise crop losses and to aid capacity-building for sustainable agriculture and rural development in active volcanic regions ([10]; [42]). The present work, thus, aims to assess experimentally the physical impact of volcanic ash on rice growth in relation to morpho-physiological and biochemical parameters under controlled environmental conditions.

6.3 MATERIALS AND METHODS

6.3.1 *Plant material and growing conditions*

Seeds of *Pokkali* were purchased from IRRI (International Rice Research Institute; Philippines). The 25 rice seeds were placed in Petri dishes with one sheet of filter paper, to which 10 ml of demineralized water was added. Seeds were incubated in a germination room at 30 ± 1 °C for 13 days with a 12-h light/12-h dark photoperiod. Thirteen days after germination, rice seedlings were transplanted to 1.5 L plastic pot (two seedlings per pot) filled up to 1.3 L with a hydroponic solution (pH 5.8), prepared according to [47]. Solutions were renewed twice per week. Plants were anchored to perforated floating polystyrene plates (11.5×16 cm), floating on top of the solution. The

experiment was performed in a phytotron room under a 16 h photoperiod. Light intensity was maintained between 210 to 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ using fluorescent lamps (Master TL-D reflex Super 80 58W/840, Philips, Brussels, Belgium). The day and night temperature was maintained at 25 ± 2 °C and 22 ± 1.5 °C, respectively. Relative humidity was 65-75%.

6.3.2 *Ash treatment*

Ash from the 1991 eruption of Mt. Pinatubo volcano, Philippines, was used in all experiments (Fig. 5.1). Ash deposition on rice plants was simulated after one week of seedling adaptation to the hydroponic solution. The ash particle size distribution estimation (Fig. D.1) and the formation of an ash deposit on the adaxial rice leaf surfaces (Fig. 5.2) were performed so as described in the section “Materials and Methods” of Chapter 5. An attempt was made to acquire a uniformly distributed ash deposit.

Two scenarios of ash deposition on the rice plants were considered: (i) a one-event scenario (S1) in which 180 g of ash were applied once (Fig. 6.1a), and (ii) a multiple-event scenario (S2) in which 60 g of ash were applied three times (i.e. on days 0, 3 and 8 after transplanting seedlings to hydroponic solutions) (Fig. 6.1b). The experiments lasted two weeks and involved six replicates (i.e. total of 48 ash-affected plants). The control consisted of 48 plants that did not receive ash.

6.3.3 *Leaf temperature, photosynthesis-, plant growth-related and biochemical parameters*

Leaf temperature, photosynthesis- and plant growth-related parameters were monitored eight times throughout the experiment on days 1, 2, 3, 4, 7, 9, 11 and 14 after the first ash application. The first ash application was performed in the beginning of the tillering stage, when the

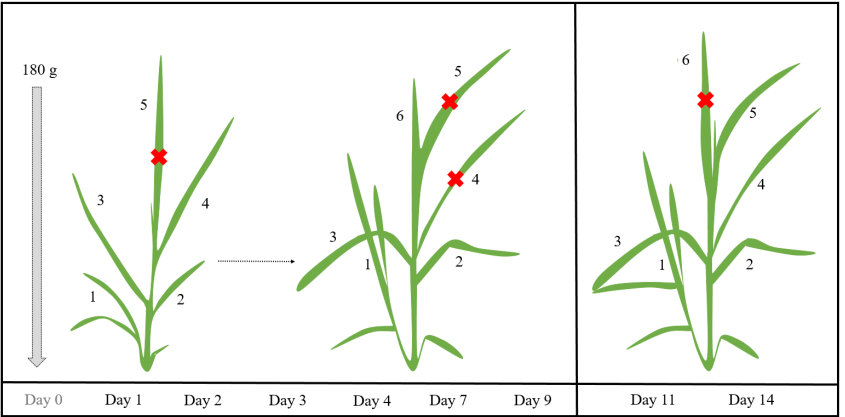
main culm bore five leaves (Fig. 6.1). Leaf temperature, photosynthesis- and plant growth-related measurements were taken on fully expanded leaf “n° 5” (numbered acropetally) on days 1-4, 7 and 9. Subsequent measurements were performed on leaf “n° 6”. Thus, leaf “n° 6” was never exposed to ash in the S1 scenario, whereas it received ash on days 3 and 8 in the S2 scenario. The measurements of the

- leaf temperature (T),
- photosynthesis-related parameters (net photosynthesis (A); inter-cellular CO_2 concentration (c_i); instantaneous transpiration (E); water use efficiency (WUE); the maximal efficiency of $PS2$'s photochemistry in the dark-adapted state (F_v/F_m); photochemical quenching coefficient (qP); non-photochemical quenching (NPQ); actual $PS2$ efficiency (Φ_{PS2}),
- plant growth-related parameters (shoot height (H); root length (L); shoot dry weight (DW_s) and root dry weight (DW_r), shoot water content (WC_s) and root water content (WC_r)),
- biochemical parameters (total chlorophyll content ($(chl\ a + chl\ b)$); carotenoid content ($Ccar$); sugars; proteins; malondialdehyde (MDA); protein-bound carbonyl and total antioxidant activity (hydrophilic (HAA) and lipophilic (LAA) fractions)
- and statistical analyses

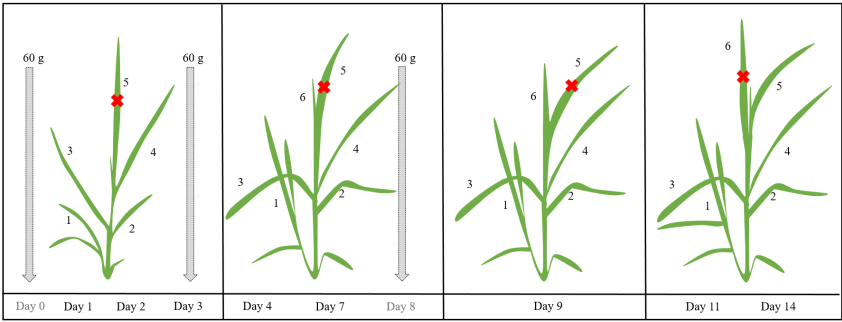
were performed as described in the section “Materials and Methods” of Chapter 5.

6.4 RESULTS

Almost all measured parameters (plant-growth related, photosynthesis- and leaf temperature), except root length and stomatal conductance, differed significantly with time (plant age) and between treatments (Table E.1).



(a) S1 scenario



(b) S2 scenario

Figure 6.1: Scenarios of ash application corresponding to one single event (a, S1) or to a multiple-event (b, S2). In scenario 1, plants received 180 g of ash on day 0, while in scenario 2, plants received 60 g of ash on days 0, 3 and 8. Leaf temperature, photosynthesis- and plant-growth-related measurements were performed 8 times during a two-week treatment. The position of these measurements is indicated with a red cross and corresponds to a leaf n° 5 (numbered acropetally) for day 1 until day 9, and to a leaf n° 6 for days 11 and 14. Hence, leaf n° 6 appeared after the ash treatment in scenario 1, while it received two ash applications in scenario 2.

6.4.1 *Plant growth and development*

Visually, the parts of leaves covered by ash became lighter in colour compared to the control leaves within four days after ash application (Fig. E.1). In addition, individual leaves were pinned down to the ground, broken, rolled and/or wilted. The impact was more severe on the S2 leaves. The newly grown leaves of the treated plants, not covered by ash, grew straight and looked healthy. The reduction of shoot height (H) of ash-treated plants on the first four days of experiment was, on average, 21.5% (Fig. 6.2). The shoot height of the plants in S1 and S2 remained on average 21% and 30% lower, respectively, than that of the control plants on days 7, 9, 11 and 14. In contrast, ash did not affect the root length (L) of treated plants across the whole period of experiment. The DW_s of the plants in S1 and S2 decreased on the third day and remained up to 46% lower than that of the control plants until the end of experiment (Fig. 6.3). Ash application had only marginal effect on DW_r of the plants in S1 and S2 on the first four days, but drastically reduced DW_r on the following days (by 24% and 40%, respectively) (Fig. 6.3). The WC_s was slightly higher in the ash-treated plants on the first days of experiment (Fig. 6.4). Starting from the day 7, this difference increased more than twofold. The WC_s was similar for the plants in S2 and S1 scenarios. The WC_r of the ash-treated plants was also higher compared to that of the control plants.

6.4.2 *Photosynthesis-related parameters*

Ash deposition on the rice leaves did not alter F_v/F_m , $\Phi PS2$ and qP values (measured on days 4 and 14) (Table 6.1). However, non-photochemical quenching (NPQ) slightly increased in the S2 plants compared to the control plants on day 4, but not on day 14. Total chlorophyll ($(chl\ a + chl\ b)$) and carotenoid ($Ccar$) concentrations decreased by 42-48% in response to S1 and S2 ash treatments on days 1-4. The difference in $(chl\ a + chl\ b)$ and $Ccar$ concentrations between the ash-treated plants and

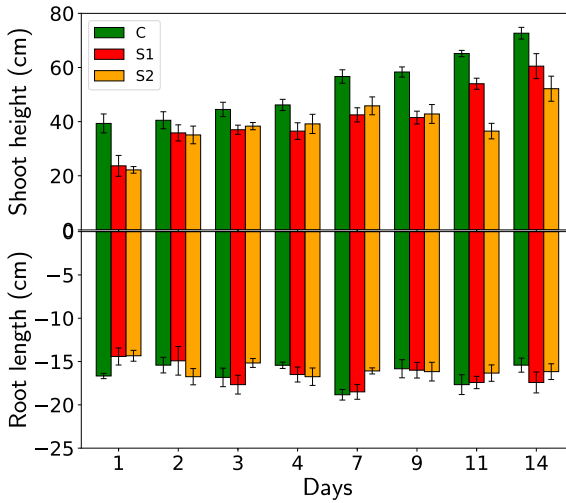


Figure 6.2: The height of the rice shoots, measured from the interface of roots and shoots up to the highest leaf, and the length of the rice roots. Results are the means of six sample replicates. Error bars represent SE from the means.

the control plants became insignificant on days 7, 9, 11 and 14.

The ash deposit on the surfaces of rice leaves led to the decreased A values (Fig. 6.5a) over the entire experimental period. The only exception was observed for S1 plants on days 11 and 14, when there was no difference between A of these plants and that of the control plants. Intercellular CO_2 concentration (c_i) in the ash-treated plants was slightly higher in the beginning of experiment, but was not different from that in the control plants in the second week of experiment (data are not shown). In S1, leaf transpiration (E) was always the same or higher than that of the control plants (Fig. 6.5b). Similar results were obtained for the plants in S2, except day 14, when E was drastically reduced

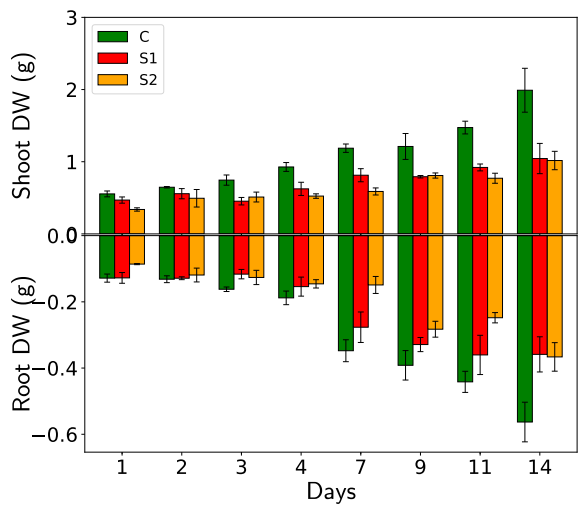


Figure 6.3: Biomass of dry rice shoots and roots. Biomass is the mean of three sample replicates. Error bars represent SE from the means.

compared to that in the control plants. Stomatal conductance (g_s) of ash-treated plants was lower than in the control plants on the first three days of experiment. In contrast, on the following days, g_s values for the plants in S1 and S2 were the same or higher than the values obtained for the control plants (Fig. 6.5c). Accordingly, WUE was lower in ash-treated plants than in the control plants on every day of measurement, except day 14 (Fig. 6.5d). The difference between WUE of the control plants and WUE of the plants in S1 and S2 ranged between 4% and 55%, and from 7 and 52%, respectively.

Finally, a constantly higher temperature (T) of the leaves in S1 was recorded on every day of measurement except day 14. In S2, T was always higher than in the control plants (Fig. 6.6; Fig. E.2). The temperature of S2-treated leaves was slightly higher, compared to that of

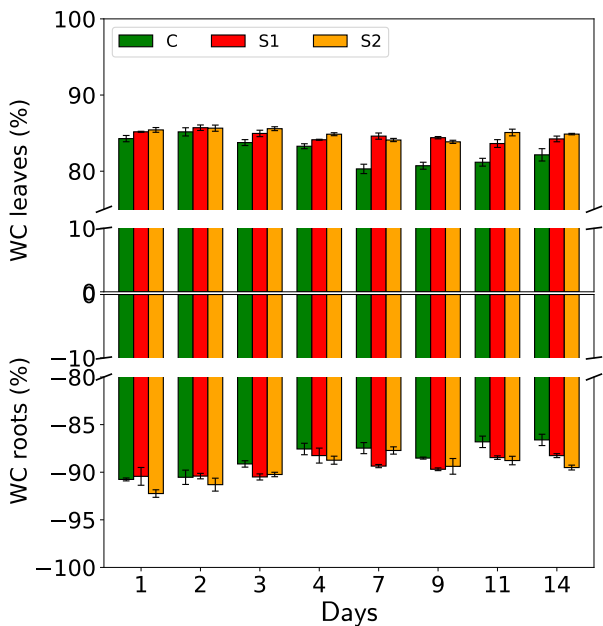


Figure 6.4: Water content in rice shoots and roots. Water content is the mean of three sample replicates. Error bars represent SE from the means.

S1-treated leaves on days 7, 9, 11 and 14. Overall, the difference between the temperature of the control leaves and that of the leaves in S1 ranged between 1% and 12%, while this difference for the leaves in S2 was in the range of 2 and 17% throughout the entire period of experiment.

Table 6.1: Photosynthesis-related parameters measured on days 4 and 14 after the first ash application. The results are the mean $\pm$ SE of six sample replicates. Chlorophyll and carotenoid contents are the mean $\pm$ SE of three sample replicates from days 1 – 4 and days 7, 9, 11 and 14.

	day 4			day 14		
	C	S1	S2	C	S1	S2
F_v/F_m	0.86 $\pm$ 0.00	0.87 $\pm$ 0.00	0.87 $\pm$ 0.00	0.85 $\pm$ 0.00	0.85 $\pm$ 0.00	0.85 $\pm$ 0.00
$\Phi PS2$	0.81 $\pm$ 0.00	0.83 $\pm$ 0.01	0.82 $\pm$ 0.00	0.82 $\pm$ 0.01	0.80 $\pm$ 0.00	0.81 $\pm$ 0.01
qP	0.97 $\pm$ 0.01	0.96 $\pm$ 0.01	0.97 $\pm$ 0.01	0.98 $\pm$ 0.00	0.97 $\pm$ 0.01	0.98 $\pm$ 0.01
NPQ	0.14 $\pm$ 0.02	0.16 $\pm$ 0.02	0.21 $\pm$ 0.03	0.13 $\pm$ 0.01	0.14 $\pm$ 0.01	0.15 $\pm$ 0.02
$(chl\ a + chl\ b), (mg\ g\ FW^{-1})$	1.38 $\pm$ 0.06a	0.72 $\pm$ 0.02c	0.79 $\pm$ 0.06bc	1.03 $\pm$ 0.01b	0.88 $\pm$ 0.08bc	0.87 $\pm$ 0.04bc
$Ccar, (mg\ g\ FW^{-1})$	0.39 $\pm$ 0.02a	0.21 $\pm$ 0.01cd	0.23 $\pm$ 0.02d	0.32 $\pm$ 0.00b	0.26 $\pm$ 0.02bc	0.27 $\pm$ 0.01bc

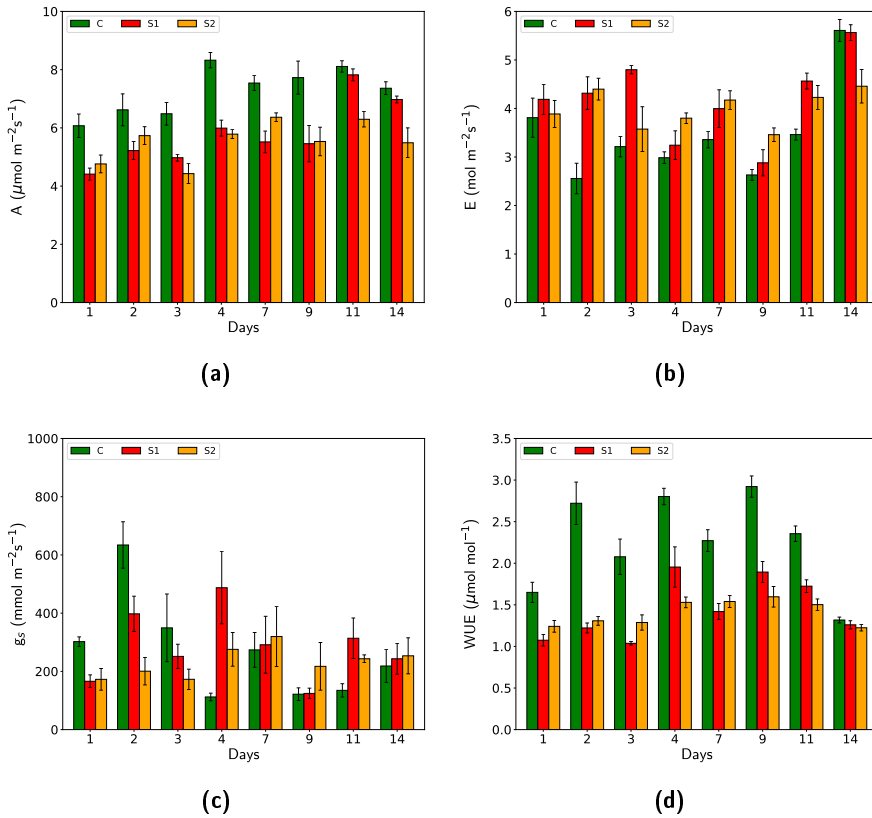


Figure 6.5: Net photosynthesis (a); instantaneous transpiration (b); stomatal conductance (c) and water use efficiency (d) of the controls, S1 and S2 leaves. Results are the means of six sample replicates. Error bars represent SE from the means.

6.4.3 Soluble sugars and protein content

There was no significant difference between total soluble sugar content in the shoots of S1 plants and that in the control plants on days 1-4 and

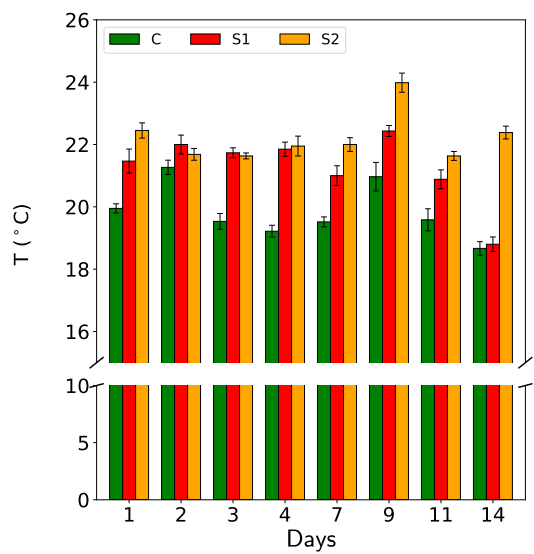
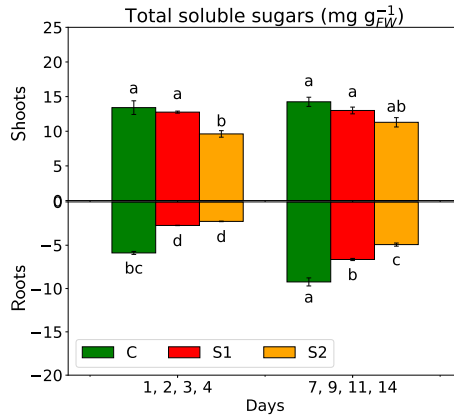


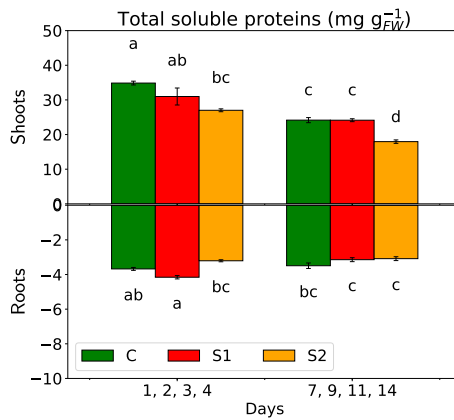
Figure 6.6: Temperature of the fully expanded leaves, obtained by IR-camera. Leaf temperature is the mean of six sample replicates. Error bars represent SE from the means.

7, 9, 11 and 14 (Fig. 6.7a). In contrast, sugar content in the shoots of S2 plants was substantially (28%) lower than in the control plants on days 1-4, while it was not significantly different from the control plants on days 7, 9, 11 and 14. The average sugar content in the roots of the control plants was more than twofold the concentration in the roots of plants in S1 and S2 on days 1-4. This difference decreased to 28% and 47% in the roots of S1 and S2 plants, respectively, on days 7, 9, 11 and 14.

There was no significant difference between protein content in the shoots of S1 plants and that in the control plants on days 1-4 and 7, 9, 11 and 14 (Fig. 6.7b). In contrast, protein concentration in the shoots of S2 plants was 23-26% lower on days 1-4 and 7, 9, 11 and 14. Ash



(a)



(b)

Figure 6.7: Total soluble sugar content (a) and total soluble protein content (b) in the controls, S1 and S2 rice shoots and roots on days 1 – 4 and days 7, 9, 11 and 14. Results are the means of three sample replicates. Error bars represent SE from the means. Means with different letters are significantly different (Tukey's test, $p < 0.05$).

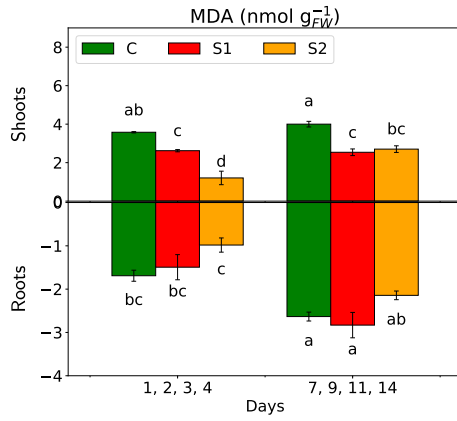
treatment did not affect significantly the protein content in the rice roots.

6.4.4 *Oxidative stress-related parameters*

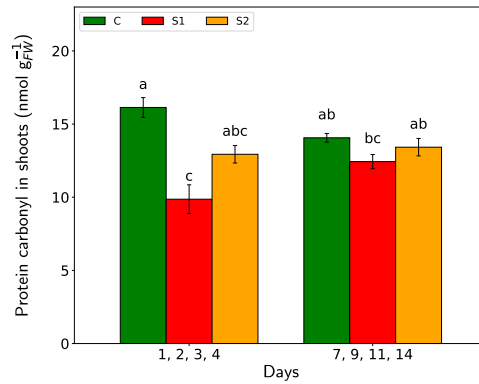
A decrease in the shoot malondialdehyde (*MDA*) concentration following ash deposit was observed on days 1-4 and 7, 9, 11 and 14 (Fig. 6.8a). The recorded 66% *MDA* decrease in the shoots of S2 plants was about twofold the decrease in the shoots of S1 plants on days 1-4. This difference lowered for the shoots of the plants in S1 and S2 on days 7, 9, 11 and 14 (37% and 32%, respectively). There was no significant difference between *MDA* content in roots of the ash-treated and that of the control plants on days 1-4 and 7, 9, 11 and 14.

The content of protein carbonyl groups in the shoots of the plants in S1 was lower than that in the control plants by 39%, while there was no significant difference between the protein carbonyl content in the control plants and in the shoots of the plants in S2 on days 1-4 (Fig. 6.8b). Similarly, there was no difference in the content of protein carbonyl groups between the shoots of the control plants and shoots of the ash-treated plants on days 7, 9, 11 and 14. The content of protein carbonyl groups in the roots of the control plants was 73% higher than in the roots of the S2 plants and was not significantly different from that in the roots of the S1 plants on days 7, 9, 11 and 14 (data not shown).

The hydrophilic antioxidant activity (*HAA*) values are presented in Figure 6.9. The *HAA* values in the shoots of the control plants were up to 30% higher than those measured in the shoots of the ash-treated plants during the entire period of experiment. In contrast to *HAA*, the lipophilic antioxidant activity (*LAA*) values in the shoots of the plants in S1 were similar to those in the control plants on days 1-4 and 7, 9, 11 and 14.



(a)



(b)

Figure 6.8: MDA in the controls, S1 and S2 rice shoots and roots on days 1 – 4 and days 7, 9, 11 and 14 (a); carbonyl contents in the controls, S1 and S2 rice shoots on days 1 – 4 and days 7, 9, 11 and 14 (b). Results are the means of three sample replicates. Error bars represent SE from the means. Means with different letters are significantly different (Tukey's test, $p < 0.05$).

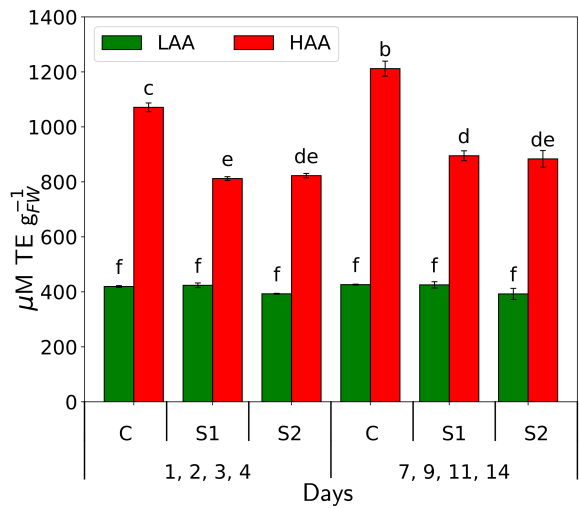


Figure 6.9: Hydrophilic and lipophilic antioxidant activity in rice shoots on days 1 – 4 and days 7, 9, 11 and 14. Results are the means of three sample replicates. Error bars represent SE from the means. Means with different letters are significantly different (Tukey’s test, $p < 0.05$).

6.5 DISCUSSION

6.5.1 *Effects of ash deposition on plant growth*

Only a part of the ash initially applied to rice plants stuck to the leaf surfaces during the entire duration of the experiment (i.e. two weeks). Considerable amount of ash was lost, which resulted in partial leaf coverage by ash. This emphasises the important role of leaf position and characteristics in determining the ash residence time on vegetation. Rice has glabrous leaves and, in contrast to pubescent leaves, is less prone to retain solid particles such as ash on its foliage ([9], [21], [46])

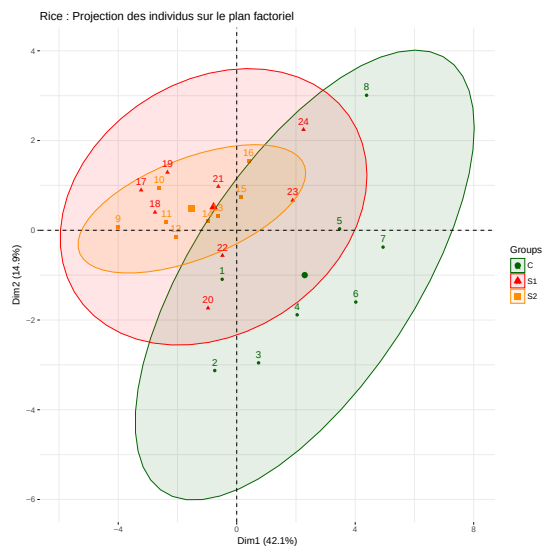
and dust of non volcanic origin ([40]; [30]). Rice leaves grow vertically, which may obstruct particle retention. Leaf bending due to weight of the ash deposit occurred in response to the single ash deposition event (S1). The leaves were also tilted in the multiple ash deposition scenario (S2), but it heavily impacted only the oldest leaves that had been exposed to more than one ash treatment. This observation is in accordance with the previous field studies, reporting the positive correlation of the magnitude of plant damage with the amount of ash on the foliage and periodicity of ash depositions (e.g., [16]; [5], [36]; [25]).

Along with the observed foliar damages, including breakage, wilting and rolling, the decrease in rice dry weight (DW_s) and height (H) measured in both the S1 and S2 experiments (Figs. E.1 and E.2) strongly suggests a reduction in plant photosynthesis ([19]). Similarly, a strong decrease in plant growth parameters, such as biomass and plant height, accompanied by lower photosynthesis has been reported for plants exposed to cement (kiln) dust ([14]; [38]; [31]; [1]). After the 1980 eruption of Mt. St. Helens, [9], [36]; [16] and [21] reported decreased growth, development of diseases and premature leaf abscission and fruit drop in plants affected by ash falls. ([9]) posited that the long-term presence of ash on leaves hampered photosynthesis and damaged the protective waxy cuticle of leaves, thereby lowering their resistance to stress. More recently, [46] proposed that leaf damages, such as shrivelling and wilting, on tomato plants exposed to ash emissions from the 2006 eruption of Mt. Merapi were primarily due to the release of acidic ash leachates. However, this type of chemical interaction does not apply here as the rice leaves were never in contact with liquid water. In addition, the ash material used in our study was exposed to rainwater prior to collection and therefore, the salts that may have been present on the ash surfaces had long been dissolved.

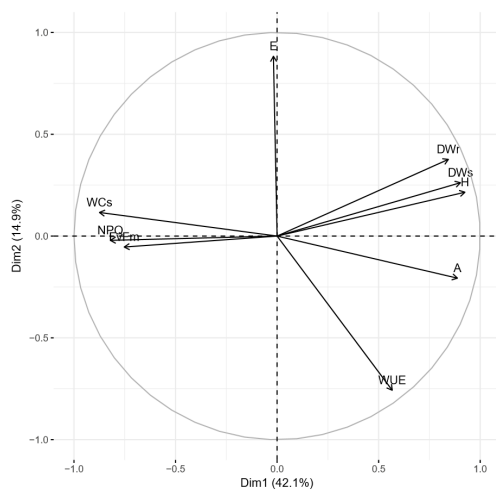
6.5.2 *Effects of ash deposition on plant physiology and biochemical parameters*

Ash on rice foliage induced various changes in the 16 parameters (i.e. A , E , g_s , c_i , T , WC_r , WC_s , WUE , DW_r , DW_s , H , L , F_v/F_m , $\Phi PS2$, qP , NPQ) that were tested by PCA for assessing plant growth and the condition of the photosynthetic system. Figure 6.10a shows the coordinates of the points corresponding to the control, S1 and S2 plant groups. The position of the S1 group is shifted with regard to the position of the control group, but individual variants are overlapping. This suggests that there are enough features that can be used for distinguishing the different treatments, despite similarities in the growth and physiological parameters between the control plants and the plants in S1. The S2 group lies in the narrower region compared to the S1 group, and it is well separated from the control group. This points to a higher effect of several ash additions on plant growth and physiological parameters. The vectors of the considered variables (plant growth and physiological parameters), presented in Figure 6.10b, show that the first principal component (Dim1) is positively correlated with DW_r , DW_s , H and A . This suggests that these four parameters vary together. At the same time, the first principal component is negatively correlated with WC_s , F_v/F_m and NPQ and therefore, with DW_r , DW_s , H and A . The second principal component (Dim2) shows the negative correlation of E and WUE .

The chlorophyll fluorescence analyses (Table 6.1) show that the photosynthetic machinery of S1 and S2-treated plants was not damaged during the duration of experiment. Indeed, $\Phi PS2$, qP and F_v/F_m values in S1 and S2 plants did not differ from those in the control plants. This suggests that no damage to photosystem $PS2$ occurred and that ash-treated plants were able to perform light-phase of photosynthesis when ashes were removed from the leaf surface, as was the case in our experimental conditions (see Materials and Methods section). Similar to our result, [27] did not observe the substantial decrease in photochemical efficiency (F_v/F_m) of *Avicennia marina* leaves covered by



(a)



(b)

Figure 6.10: Principal Component Analysis of variability of parameters, tested for assessing growth and physiological condition of the controls and ash-treated plants (S1 and S2) (a); Vectors of the considered variables showing the correlation between them (b).

coal dust and suggested that no severe photoinhibition occurred in the dusted leaves. Although, there was no changes in chlorophyll fluorescence parameters, lower chlorophyll concentrations were measured in ash-treated plants on days 1-4. This result confirms the previous work, showing that the relationship between chlorophyll content and chlorophyll fluorescence is ambiguous ([41]). However, on days 7, 9, 11 and 14 chlorophyll content in the ash-treated plants increased and did not differ from that measured in controls, suggesting the plant recovery.

Non-photochemical quenching (*NPQ*) usually acts as a safe mechanism for the dissipation of excess energy as a heat ([26]). In our experiment, the slightly increased *NPQ* in S2 plants on day 4 may have been mediated by a particular group of carotenoid pigments of the xanthophyll cycle ([12]; [8]). However, the total carotenoid content in the ash-treated plants decreased on days 1-4 (Table 6.1). Usually the xanthophyll cycle is activated under light excess ([12]; [8]). In our experiment, the leaves were not exposed to light excess but rather to a light deficit due to the presence of the ash deposit. While it is difficult to conclude about the mechanism responsible for the observed variations in *NPQ* on day 4 of the S2 ash treatment, the observed modifications were transient: on the following days, ash treatment had no impact on *NPQ* and carotenoid content. This suggests that repairing and adaptive mechanisms were efficiently operating at the photosystem levels, and in both ash deposition scenarios the leaves were able to perform the light-dependent phase of photosynthesis.

Net photosynthesis (*A*) decrease was observed throughout the entire experimental period in the ash-treated plants, except S1 plants on days 11 and 14 (Fig. 6.5a). Interestingly, almost complete inhibition of photosynthesis was quoted for apple leaves covered with 1 mm of Mt. St. Helens ash ([9]). It was also demonstrated that particles of ash less than 2 μm clogged the leaf stomata, impeding gas exchange and hence, affecting the leaf temperature and photosynthesis ([16]; [18]). Similarly, investigations on the effect of deposition of road, industrial

and cement dusts on vegetation highlighted perturbations of stomatal conductance (g_s) due to blockage by particles ([35]; ([37]; ([14])). In our experiment, photosynthesis reduction could be attributed to the decrease in g_s during the first three days of experiment. However, in the following days, inhibition of photosynthesis probably did not relate to stomatal conductance limitation, as there was no evidence of stomatal closure in response to ash treatment (Fig. 6.5c). This can be explained by the fact that the simulated ash fall formed a deposit on the adaxial side of rice leaves. Since stomata in rice leaves are equally present on both the adaxial and abaxial sides ([29]), ash particles were not in close contact with guard cells located on the abaxial side. Thus, there was only partial physical interference of the ash particles with stomatal regulation. Another explanation of limited stomatal closure in the S1-treated plants is that the new leaves, appearing on the course of plant growth, were not exposed to ash treatment and, therefore, no stomata closure due to physical ash impact could have occurred.

A decrease in leaf photosynthesis (A) likely explains the lower protein and sugar concentrations determined in the S1 and S2 plants ([31]; [32]). Notably, DW_r was especially reduced in the ash-treated plants, although ashes were applied only on the leaves and plants maintained in a nutrient solution that was not in contact with ash. The decrease in root sugar concentration upon ash treatment (Fig. 6.7a) may account for reduced sugar translocation from the shoot to the root system. In natural conditions, a decrease in root growth would have a severe impact on nutrient and water absorption [43].

Although no obvious water stress was reported in our material, we recorded an increase in transpiration (E) values occurring concomitantly with low stomatal conductance (g_s) on the first three days (Fig. 6.5). These apparently contradictory data strengthen the hypothesis of an ash-induced increase in cuticular transpiration ([27]). However, our data obtained after 4 days of experiment show the same or slightly higher g_s and E values for the ash-treated plants than for the control

plants. This result may support our assumption that guard cells were not completely clogged by ash particles and their function was not depressed. Alternatively, this could suggest that ash-treated plants have responded to the new conditions created by the ash deposit, for example, by changing the number of stomata, which could have helped to regulate the transpiration and to limit possible entry of particles into plant tissues ([31]). The fact that the new leaves, not covered by ash, were used for the measurements in S1 on days 11 and 14 could explain no evidence of lower g_s and E values in S1 plants compared to those in the control plants. The overall consequence of a decrease in A values and an increase in E was a decrease in WUE . Although WUE was not a limiting factor in hydroponic culture in our experiment, it may lead to serious drought symptoms in field conditions ([48]).

Infrared thermography measurements have been successfully used to monitor leaf stomatal conductance (e.g., [17]; [49]). However, our data do not show the clear relationship between the leaf temperature and g_s values of the ash-treated plants (Fig. 6.5). Light interception by an ash deposit can lead to the increased leaf temperature ([36]) and/or photosynthetic activity indirectly by lowering the quantity of radiation reaching the leaf chloroplasts ([16]). The shading effect due to dust particle accumulation on the leaves has also been reported to reduce leaf chlorophyll concentration and therefore, photosynthesis (e.g., [38]; [33]). Thus, ash particles from S1 (on days 1-4, 7 and 9) and S2 (on days 1-14) on rice leaves act as a physical barrier. This effect probably explains both the decrease in photosynthesis and increase in leaf temperature. Moreover, as in S1 scenario leaves were not exposed to the ash treatment on days 11 and 14, the shading effect may explain the higher leaf temperatures on these days in S2 application scenario.

The MDA and carbonyl residues in the ash-treated plants were not higher than in the control plants (Figs. 6.8a and 6.8b). These compounds are typically produced by oxidative damages of membrane lipids and protein, respectively. This result is rather surprising, as

generation of ROS in plants subjected to various environmental stress conditions has been widely documented (e.g., [11]). Our findings may reveal an efficient antioxidant defensive mechanism to combat oxidative stress. However, such mechanism is not compatible with lipophilic unaltered antioxidants (LAA) and reduced hydrophilic antioxidant concentrations (HAA) measured after ash treatment (Fig. 6.9). Alternatively, the shading effect of the ash coating on the leaf surface may be responsible for the subdued MDA and carbonyl residue concentrations. It is well known that chloroplasts are major sources of ROS ([24]; [11]). The amount of light that could reach chloroplasts in the ash-coated leaves may have been reduced to such extent that oxidative stress, and relatedly MDA and carbonyl residue production, was drastically hampered. This suggestion is partly supported by higher ROS generation in the control rice plants compared with the ash-treated ones (Fig. 6.9).

6.5.3 *Single versus multiple ash deposition*

The rice plants responded differently to ash deposition in S1 and S2. On the first 4 days of experiment, the higher NPQ values and the lower sugar and protein contents in the S2-treated plants compared to those in S1-treated plants indicate the higher sensitivity of the rice plants to the multiple ash deposition scenario, rather than to a single ash deposition scenario.

The more deleterious effect of S2-treatment on photosynthetic activity, leaf temperature, root biomass, sugar and protein contents on days 7, 9, 11 and 14 also emphasizes the significance of the repeated ash additions in plant functioning.

This difference in photosynthesis and leaf temperature noticed between the S1 and S2 plants may relate to the fact that the temperature and physiological measurements in the end of the experiment (days 11 and 14) were carried out on leaves “n° 6”. As already mentioned

in section 2.3, leaves “n° 6” did not receive ash in S1, but they did in S2.

The values obtained for the net photosynthesis for leaves “n° 6” in S1 were similar to those measured for the leaves in the same position on the control plants (Fig. 6.1). This would imply that ash deposited by a single event on rice plants did not cause a knock-on effect on new organs. Most likely, ash losses after S1 caused by inability of the glabrous vertically-positioned leaves to keep ash for a long time allowed for the further rice growth and production of new healthy leaves, which helped fast rice recovery. However, the drastically lower shoot (DW_s) and root (DW_r) biomasses in both S1 and S2 suggest that a single ash application was enough to affect the entire plant viability.

Most probably, more time would be needed for plants to adapt to the new conditions and completely overcome this stress. Our results demonstrate not only the mechanical rice damage by ash depositions, but also shed light on the physiological and biochemical rice processes occurring in the first two weeks after ash application.

6.6 CONCLUSIONS

The present study investigated a single and a multiple event of ash deposition on rice leaves in controlled environmental conditions. The plant morphological, photosynthesis-related and biochemical parameters were monitored during two weeks. We assume that severity of the initial mechanical damage of rice is dictated by the amount of ash on the leaves, while the magnitude of mechanical impact over the longer time depends on the frequency of ash deposition. Further, our findings show the ability of rice plants to overcome unfavourable conditions within the first days regardless the mechanical damage by a single ash load. In contrast, the multiple ash loadings on foliage directly cause more detrimental impact on rice biochemical parameters (i.e. sugar and protein contents). The ash effect on the growth and

photosynthesis-related parameters (i.e. net photosynthesis, leaf temperature, root biomass) is amplified in the second week of treatment. We suggest that the quantity of radiation reaching the leaf chloroplasts of the ash-treated leaves was reduced through the shielding effect of the deposit. This probably explains the observed decreased in photosynthetic activity, which, in turn, led to lower sugar contents and reduction in plant growth. Unexpectedly, neither an oxidative damage nor an increase in antioxidant activity could be evidenced in the ash-treated plants. The reduced amount of light captured by the leaf chloroplasts may have strongly hampered development of an oxidative stress. Further studies will improve our understanding of ash effects on plants and their mechanisms, with a particular emphasis on plant recovery.

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Part III

SUMMARY AND PERSPECTIVES

CONCLUSIONS AND PERSPECTIVES

7.1 MECHANISMS OF SURFACE CRUSTING OF AN ASH DEPOSIT

The study presented in Chapter 3 provides new insights into the mechanisms responsible for crust formation in the surface of an ash deposit (Fig. 7.1). Three laboratory-constructed ash deposits were subjected to simulated rainfall under controlled conditions. Formation of a structural crust in the vicinity of the deposit surface occurred in all cases. Crusting is governed primarily by ash particle reorganisation in response to raindrop impact, i.e. it was not due to chemical cementation of particles. The crust was typically comprised of a thin layer of tightly packed clay and silt-size ash particles, sometimes overlain by loose coarser materials. The thin layer exhibited a significantly lower porosity compared to bulk soil. Different crust formation mechanisms were identified depending on the particle size distribution of the ash sample. A deposit of fine-grained ash will probably rapidly form a crust in response to compaction and eluviation/illuviation mechanisms. Compaction may dominate when coarser material is also present in the ash deposit. For even coarser textures, water percolation is mainly responsible for particle sieving and eluviation/illuviation. However, in this case, surface crusting is less developed and discontinuous.

Overall, our study provide novel insights for interpreting post-deposition crusting of a natural ash deposit. Future experiments similar to those designed and developed here could encompass a broader range of ash types and rainfall parameters. In particular, ash materials with contrasted particle size distributions could be tested to confirm this parameter as a key control on ash crusting. As the duration and magnitude of effects of ash on the soil-atmosphere and leaf-atmosphere

depend on the residence of ash, the strength of the ash crust could be assessed in relation to wind- and water-driven erosion intensity. It would also be interesting to investigate how the ash crust evolves when subjected to several wetting-drying cycles. Further, raindrop size and rainfall intensity have been shown to partly control the development of structural crusts in soils (e. g., [4]). The same may apply to ash crust formation. Finally, the influence of an ash crust on seed germination could be studied in the laboratory. By doing more experiments, we will increase our understating of how the surface properties of a volcanic ash deposit subjected to rainfall evolves with time. This has important ramifications for predicting the effects of an ash layer on agricultural soils, run-off and lahar initiation.

7.2 COLONIZATION OF A COARSE-GRAINED TEPHRA DEPOSIT BY FUNGI AT MT. ETNA

Chapter 4 reports on the rapid colonization (< 10 months) by fungi of a coarse-grained tephra deposit emplaced on the flank of Mt. Etna in 2013. Scanning electron microscopy, fluorescence, membrane sterol extraction and denaturing gradient gel electrophoresis analyses showed that colonization of fungi was favoured in the tephra layer in direct contact with the buried soil. Ectomycorrhizal fungi seem to be the dominant species that colonize the deposit. This probably reflects the fact that ectomycorrhizal fungi are usually less dependent on the organic matter content of the substrate on which they grow than saprotrophic fungi. The results of the molecular analyses confirmed the presence of at least two ectomycorrhizal fungi species, i.e. *Russula sp.* and *Pseudotomentella sp.*, which are able to associate with the surrounding *Pinus sp.* These findings suggest that colonization of tephra deposit was initiated by ectomycorrhizal fungi from underlying soil. Overall, the results of this study demonstrate that freshly-deposited tephra represents a potential ecological niche for fungi. With time, fungi growth will probably enhance tephra weathering while contributing to tephra

organic carbon accretion in the tephra. As such, fungi may be important agent of soil formation and thus, re-vegetation process, in coarse-grained tephra deposits (Fig. 7.1).

The results generated in this field study could be nicely complemented with new experimental investigations and laboratory analyses. For example, the affinity of specific fungi species for the tephra substrate could be assessed under controlled environmental conditions. The molecular techniques applied in this study have allowed to determine fungal composition in the tephra and buried soil materials. While digital gradient gel electrophoresis provided a first evaluation of fungal diversity, this technique can only detect community members with a relative abundance higher than 1 % of the total community ([5]). The deployment of higher resolution techniques such as terminal restriction fragment length polymorphism (TRFLP) and high-throughput sequencing platforms (illumina, Nanopore or PacBio) would improve the description of the fungal community that colonizes tephra immediately after its deposition. For instance, TRFLP has been shown to be a more sensitive fingerprinting approach than denaturing gradient gel electrophoresis for monitoring changes in fungal community structure in temperate grassland soils ([3]). Furthermore, it would certainly be interesting and complementary to investigate further the composition not only of fungal communities but also of bacterial communities. This would allow for better understanding of the ability of each group of microorganisms to colonize tephra.

Mt. Etna study site provides an exciting opportunity for investigating the colonization of tephra by fungi and other microorganisms. Thus, research should be continued to study the evolution of the microbial community assembly over time. This should contribute to deepen our knowledge of how communities evolve and interact between each other in response to a natural disturbance. There is also a prospect for assessing the role of fungi in the early stages of soil development, i. e. weathering and organic matter accumulation. In particular, it would be

interesting to investigate the fungal contribution to tephra weathering on the longer-term scale, the symbiotic associations (mycorrhiza) that fungi can form with most plants (in particular conifers) are particularly adapted for the colonization of silicate minerals ([2]) and more generally volcanic rocks ([6]). Through growth at the surface of minerals, fungi feed on inorganic nutrients (K, P, Mg or Fe) presents in silicate minerals ([1], [7]). In doing so, they contribute to mineral weathering and soil formation.

7.3 IMPACT OF ASH DEPOSITION ON CROP PLANTS

In Chapters 5 and 6, we studied the effects of an ash deposit on tomato and rice foliage on plant growth, morpho-physiological and biochemical parameters under controlled environmental conditions. In general, tomato and rice displayed comparable behaviours following exposure to ash. A visual summary of our findings is provided in Figure 7.2. Similar to previous field observations, we highlighted stronger mechanical plant damages at high ash loading. On the other hand, the largest effects on plant biomass, photosynthetic activity and biochemical parameters were observed when lower amounts of ash were applied repeatedly to plants. Conversely, quick (within two weeks) recovery from stress was also observed in plants treated once with ash. Oxidative damages in response to ash treatment did not occur, and accordingly no increase in the antioxidant activity was detected. This probably reflects a shielding effect of the ash layer on foliage and consequent reduction in the quantity of light that could reach the leaf chloroplasts. The lower photosynthesis activity detected in the ash-treated plants probably resulted from the same mechanism and/or an alteration of the Calvin cycle or other components of the dark phase of photosynthesis. Compared to tomato plants, rice plants seem to suffer less from ash deposition. This statement is based on the following observations: there was no decrease in photosynthetic efficiency of the rice leaves; sugar and protein contents were less affected; net photo-

synthesis, chlorophyll and carotenoid contents increased in the second week of experiment (Fig. 7.2). This may be due to differences in leaf shape and position with respect to the ground. In the case of rice, these parameters may have allowed less ash to attach to foliage.

The knowledge obtained in the present study can be complemented by additional experimental studies to investigate further ash impacts on vegetation. The different responses of rice and tomato plants to ash deposition highlights the need for additional investigations involving other plant types to reveal all possible factors affecting plant development. The experimental set up designed and used in this PhD research can be easily reproduced to achieve this. Clearly, the residence time of ash on plant leaves is a key factor in determining the level of disturbances in plant photosynthesis and perhaps other functions. It would be interesting to study the temporal evolution of ash impacts and to assess recovery over longer timescales. Plant growth stage at the time of ash deposition is also likely to be a crucial factor. Thus, experiments aimed at assessing the most vulnerable growth stage would be invaluable to guide the production of mitigation measures in the case of a volcanic eruption affecting agricultural areas. In natural conditions, the thickness, grain size, composition and colour of a volcanic ash deposit on foliage may vary between eruptions. Clearly, more work is needed to establish how ash properties determine the type and intensity of impacts on crop plants. Ideally, dose-response curves should be generated for the most common ash types and representative crop plants. This would allow the development of fragility functions that can serve to better inform volcanic risk assessment and management ([8]).

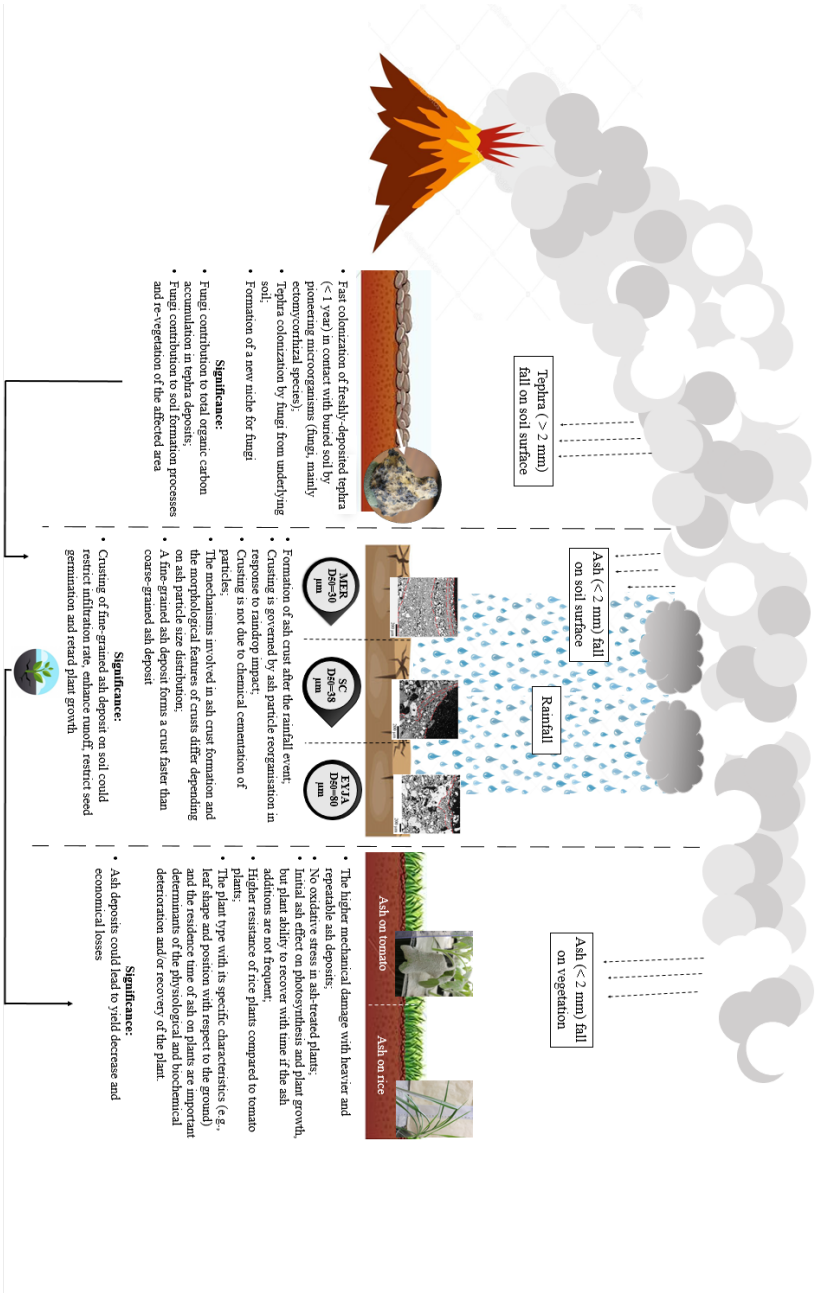


Figure 7.1: Conceptual cartoon summarising the main results on the potential of tephra (or ash) to form crust (Chapter 3), to serve as a niche for fungi (Chapter 4) and to affect plant growth (Chapters 5 and 6).

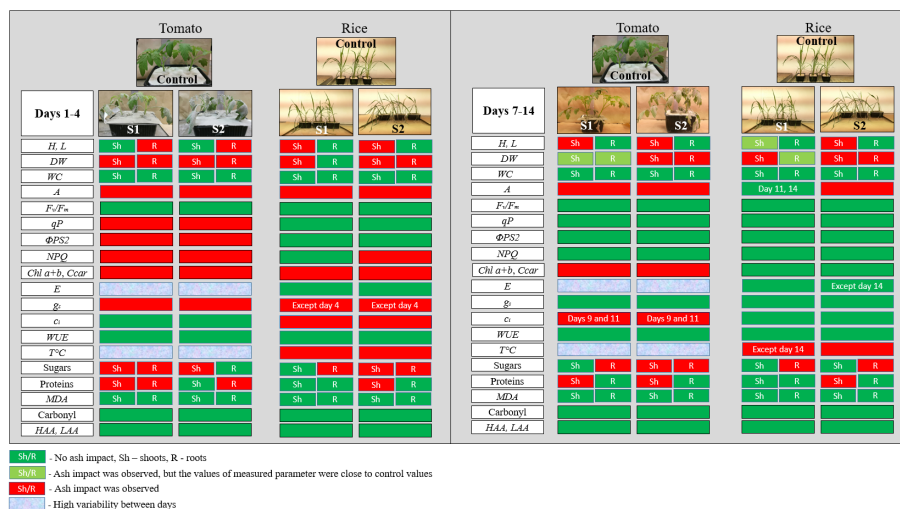


Figure 7.2: Summary of the response of the tomato and rice growth-related, physiological and biochemical parameters to ash deposition. *H* - shoot height, *L* - root length, *DW* - dry weight, *A* - net photosynthesis, *F_v/F_m* - maximal efficiency of *PS2*'s photochemistry in the dark-adapted state, *qP* - photochemical quenching coefficient, *PS2* - actual *PS2* efficiency, *NPQ* - non-photochemical quenching, *Chl a+b* - total chlorophyll, *Ccar* - carotenoid, *E* - transpiration, *g_s* - stomatal conductance, *c_i* - intercellular CO₂, *WUE* - water use efficiency, *T* - leaf temperature, *MDA* - malondialdehyde content, *HAA*, *LAA* - hydrophilic and lipophilic antioxidant activities, respectively. These parameters were monitored 8 times for 2 weeks after the 1st ash application. The results are presented for days 1-4 and 7, 9, 11 and 14. S1 and S2: a one-event scenario and a multiple-event scenario, respectively.

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Part IV

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY FOR CHAPTER 1

A.1 OBJECTIVES OF THE EU-FP7 ITN PROJECT VERTIGO

Volcanic eruptions are an unavoidable natural hazard. We will not be able, in the foreseeable future, to accurately forecast a looming eruption ("tomorrow at 10.30 a.m.") nor influence timing or eruption style. To be prepared for all eventualities of future eruptions, we must understand how volcanoes work and the possible effects are. Amongst the associated threats, ash is arguably the most far-reaching: transported over long distances and in many environments, it can harm health and society on scales local to global. At the same time, ash particles carry precious information on their origin and represent a probe to study otherwise inaccessible volcanic processes. Volcanic ash, the smallest fraction (<2 mm) of all ejected products, is a heterogeneous mixture of fragments from crystals, lava, glass and/or older rocks.

During explosive volcanic eruptions, magma rising through the crust of the Earth is broken up violently. As the fragmentation of magma into pyroclasts inside the volcano is deprived of direct surveillance, kind and efficiency of the physical processes must be unleashed from the eruptive products and laboratory experiments. Increasingly, sophisticated technology and/or degrees of resolution now allow for substantial advances in the μm -scale mapping of ash characteristics. New findings have contributed substantially to an enhanced understanding of the physical processes before and during magma fragmentation and add to a more elaborate mechanistic and qualitative understanding of explosive volcanic eruptions. Close to a volcano, ash is deposited as fall-out and as pyroclastic density currents, hot avalanches of gas, ash and larger fragments travelling down the flanks. A significant part of

ash is injected in the atmosphere (called "eruption plume" above the volcano) and is distributed far away where it can have diverse and long-lasting impact.

The objective of VERTIGO was "to improve career perspectives of Early Stage Researchers (ESRs) in both public and private sectors, thus adding to their intersectoral employability". Training-through-research projects on open questions related to volcanic ash were performed to equip the next generation of European researchers with an innovative, multidisciplinary background for careers in academia, applied research, private sector and risk mitigation entities. The VERTIGO ESRs were trained in the physical processes during explosive eruptions, the physico-chemical processes in the eruption plume and the atmosphere, numerical approaches to understand volcanic processes and identify the impact of volcanic ash on various aspects of today's and future life. Additionally, ESRs gained insights into how the private sector or other potential future employers (e. g., volcano observatories, civil protection, volcanic ash advisory centres) respond to volcanic ash related questions.

The bundle of multidisciplinary knowledge is prerequisite not only for research in modern volcanology and the adequate assessment of related hazards but will become a relevant eligibility criterion when recruiting engineers or decision-makers in private companies, volcano observatories, advisory centres, insurance companies and European politics for volcano-related problems. Apart from the connection of individual research disciplines related to volcanic ash, the close cooperation with the private sector were considered to lead to a bilateral win-win-situation: academia benefits from elaborate technology in the qualitative analysis of particulate matter and profound knowledge of volcanic ash were aimed to foster technological improvements in the private sector, farming, living or the enhanced response to volcanic ash in the daily life.

To achieve this, the ESRs aims were to (1) understand the eruption dynamics and proximal deposition of explosive volcanoes, (2) simulate explosive eruptions and proximal deposition experimentally, (3) experimentally constrain the physico-chemical processes that affect ash in the eruption plume, (4) model syn- and post-eruptive processes numerically, (5) develop tools to spatially quantify the ash concentration in the atmosphere, (6) reveal the health hazard of volcanic ash, investigate the effect of volcanic ash on (7) the growth of plants and (8) the functional reliability of airplane turbines and (9) implement all achieved data into routine procedures at the end-user (airline companies, engineering, civil protection, politics).¹

¹ Objectives of the EU-FP7 ITN project Vertigo are taken from Annexe to Vertigo contract.

SUPPLEMENTARY FOR CHAPTER 3

B.1 SAMPLE DESCRIPTION

Table B.1: Brief description of the ash samples used in the study.
a: [1], b: [2], c: [4]

Sample	Eruption date	Eruption style	Classification	Reference
MER	26/10/2010	magmatic/dome explosion	andesite	a
SC	22/01/2000	phreatic/strombolian	basalt-andesite	b
EYJA	23/04/2010	phreatomagmatic	trachyandesite	c

B.2 PARTICLE SIZE DISTRIBUTION

Particle size distribution was analysed by laser diffraction (COULTER LS 100Q). A suspension consisting of distilled water (approximately 200-250 ml) and ash (<0.5 g) was first subjected to sonication for 30 s and then passed through the analyser. The Fraunhofer dffraction equation ([3]) was applied to obtain the volumetric percentage of particles of different sizes.

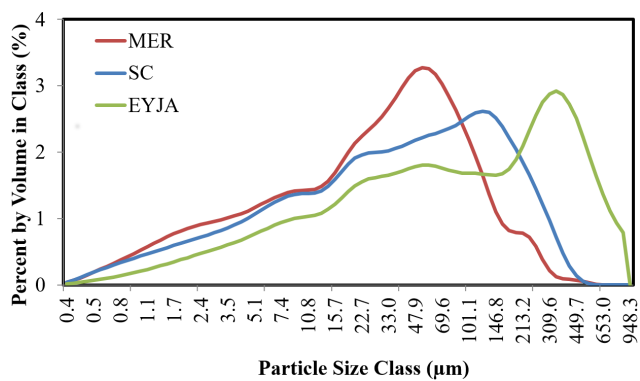


Figure B.1: Particle size distribution (wt. %) of Mt. Merapi, Indonesia (MER), San Cristobal, Nicaragua (SC) and Eyjafjallajökull volcano, Iceland (EYJA) ash samples.

Table B.2: Particle size distribution (wt. %) of MER, SC and EYJA ash samples.

Sample	clay-size (<2 μm)	fine silt- size (2-20 μm)	coarse silt- size (20-50 μm)	very fine sand- size (50-100 μm)	fine sand- size (100-250 μm)	medium sand- size (250-500 μm)	coarse sand-size (500-1000 μm)
MER	8.19	32.99	24.33	22.82	10.06	1.29	0.03
SC	6.82	30.14	18.43	18.83	20.48	5.30	0.01
EYJA	3.40	22.11	14.98	13.96	16.37	21.43	7.77

B.3 ASH LEACHATE ANALYSIS

Table B.3: Chemical compositions (in mg kg⁻¹, except TDS in g kg⁻¹) and pH of the ash leachates.

Sample	MER	SC	EYJA
pH	5.9	5.50	6.9
^a TDS	4.0	50.6	1.6
Si	20.8	42.2	19.1
Al	16.3	142.9	4.1
Fe	16.3	91.7	4.7
Mg	26	765.7	18.5
Ca	1351	14418	105.9
Na	87	87	271.4
K	41	4.8	28.6
F	17	49.9	42.7
Cl	192	2415	234.8
SO ₄	2186	32620	843

^aTDS: total dissolved solids

B.4 MICRO-TOPOGRAPHY AND MICRO-MORPHOLOGY MEASUREMENTS

Table B.4: Results of the micro-topography and micro-morphology analyses of the MER, SC and EYJA ash deposit surfaces.

Rainfall (mm)	MER				SC				EYJA			
	5	10	15	20	5	10	15	20	5	10	15	20
P_c (cm ⁻¹)	2.1 ± 0.2 ^a	1.7 ± 0.1 ^a	0.9 ± 0.1 ^b	0.8 ± 0.1 ^b	2.0 ± 0.1 ^a	1.7 ± 0.0 ^{ab}	1.5 ± 0.1 ^b	1.0 ± 0.2 ^b	-	1.5 ± 0.1 ^a	1.3 ± 0.0 ^a	1.3 ± 0.0 ^a
C_c (cm ⁻¹)	2.0 ± 0.2 ^a	1.7 ± 0.0 ^a	1.0 ± 0.1 ^b	0.8 ± 0.1 ^b	1.7 ± 0.2 ^a	1.6 ± 0.1 ^a	1.4 ± 0.1 ^a	1.0 ± 0.2 ^a	-	1.4 ± 0.1 ^a	1.3 ± 0.1 ^a	1.2 ± 0.1 ^a
R_{cp} (mm)	1.3 ± 0.1 ^b	1.9 ± 0.1 ^a	1.6 ± 0.1 ^{ab}	1.6 ± 0.2 ^{ab}	1.5 ± 0.1 ^b	1.6 ± 0.1 ^{ab}	1.8 ± 0.2 ^{ab}	2.1 ± 0.2 ^a	-	1.2 ± 0.2 ^a	1.2 ± 0.2 ^a	1.7 ± 0.3 ^a
T_{fpl} (micro-crater, mm)	0.08 ± 0.01	0.09 ± 0.02			0.05 ± 0.02	0.08 ± 0.03	0.09 ± 0.03	0.12 ± 0.04		0.28 ± 0.04	0.36 ± 0.10	0.23 ± 0.10
T_{fpl} (micro-peak, mm)	0.07 ± 0.01	0.09 ± 0.02	<i>multi-layered crust</i>		0.04 ± 0.01	0.07 ± 0.02	0.08 ± 0.02	0.12 ± 0.04	<i>0.3 based on 1 value</i>	<i>0.28 ± 0.06 when present</i>	<i>0.48 based on 1 value</i>	<i>0.39 ± 0.10 when present</i>
T_{egl} (micro-crater, mm)	0.12 ± 0.03	0.16 ± 0.05			-	-	-	0.09 ± 0.02		0.87 ± 0.20	0.69 ± 0.21	0.82 ± 0.29
T_{crust} (mm)	0.20 ± 0.03	0.25 ± 0.06	0.35 ± 0.06	0.80 ± 0.1	0.05 ± 0.02	0.08 ± 0.03	0.09 ± 0.03	0.21 ± 0.04		1.15 ± 0.20	1.05 ± 0.24	1.05 ± 0.31

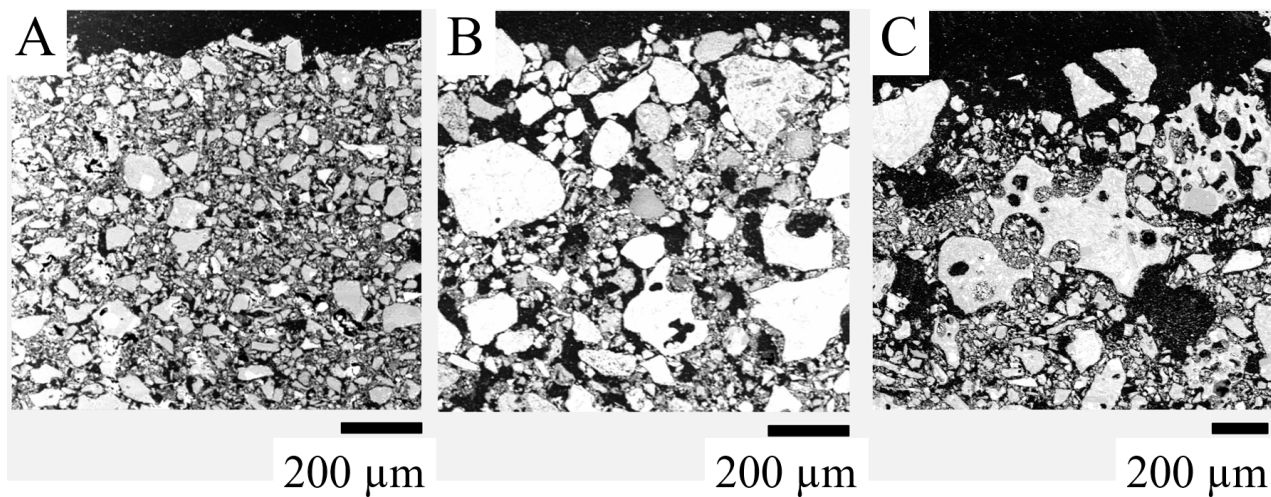


Figure B.2: Cross-sectional Scanning Electron Microscopy (SEM) images obtained for the polished sections of the MER (A), SC (B) and EYJA (C) samples protected from raindrop impact.

B.5 POROSITY MEASUREMENTS

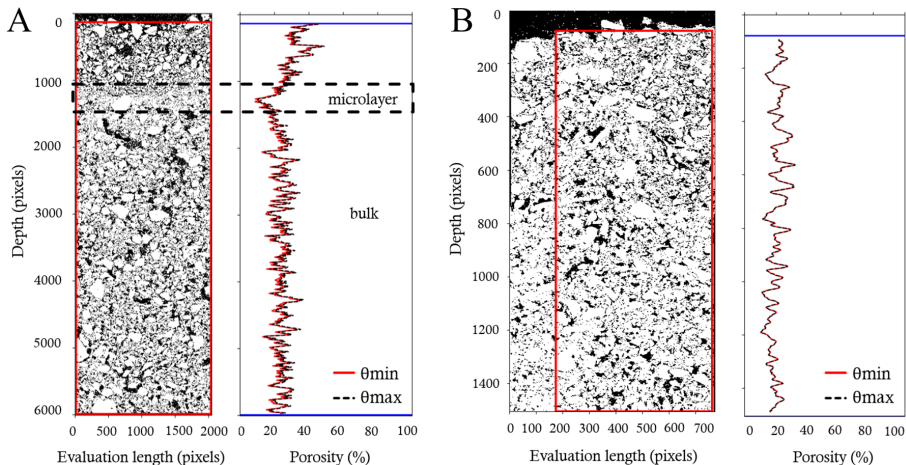


Figure B.3: Drop effect on porosity of the MER ash deposit surface exposed to 10 mm of rainfall; 1 pixel = $0.48 \mu\text{m} \times 0.48 \mu\text{m}$ (A); porosity of non-impacted by rain-drops MER ash deposit surface exposed to 10 mm of rainfall; 1 pixel = $1.28 \mu\text{m} \times 1.28 \mu\text{m}$ (B). Stitched 3-colour clustered image (left image in A and B); result of the porosity calculation (right image in A and B).

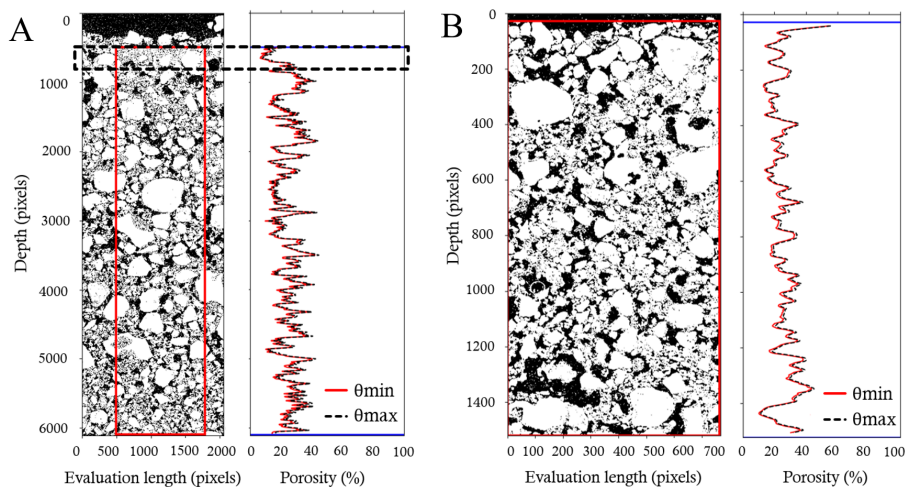


Figure B.4: Drop effect on porosity of the SC ash deposit surface exposed to 10 mm of rainfall; 1 pixel = 0.48 μm x 0.48 μm (A); porosity of non-impacted by raindrops SC ash deposit surface exposed to 10 mm of rainfall; 1 pixel = 1.28 μm x 1.28 μm (B). Stitched 3-colour clustered image (left image in A and B); result of the porosity calculation (right image in A and B).

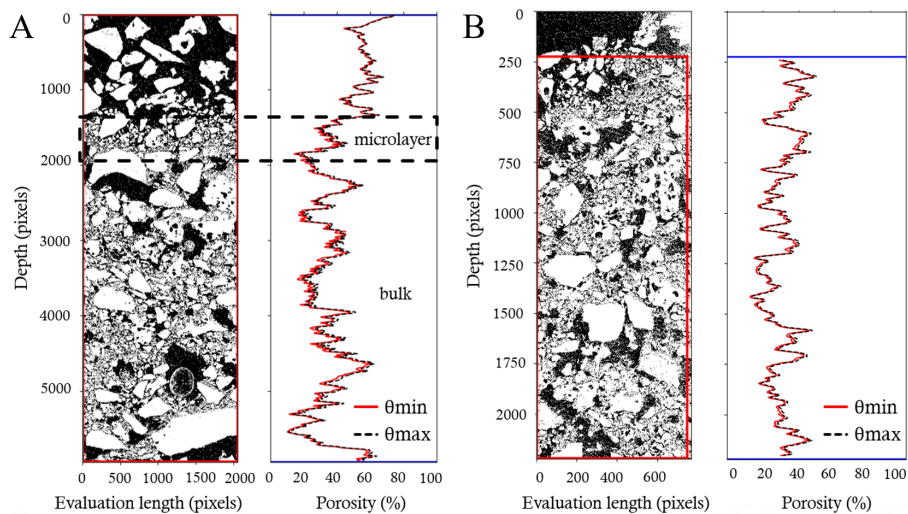


Figure B.5: Drop effect on porosity of the EYJA ash deposit surface exposed to 10 mm of rainfall; 1 pixel = 0.70 μm x 0.70 μm (A); porosity of non-impacted by rain-drops EYJA ash deposit surface exposed to 10 mm of rainfall; 1 pixel = 1.89 μm x 1.89 μm (B). Stitched 3-colour clustered image (left image in A and B); result of the porosity calculation (right image in A and B).

Table B.5: Porosity measurements: the minimum pore percentage area ($\theta_{av \min}$) values for the MER, SC and EYJA *Fine-grained layer in micro-peaks (Pfgl); Fine-grained layer in micro-craters (Cfgl); Coarse-grained layer in micro-craters (cgl)* and the bulk layer. Results are the mean $\pm$ SE of all pixel rows in the selected regions of the stitched SEM image.

rainfall (mm)	MER				SC				EYJA			
	5	10	15	20	5	10	15	20	5	10	15	20
$\theta_{av \min}$ (%)												
<i>bulk</i>	24.5 $\pm$ 0.2	29.3 $\pm$ 0.1	32.7 $\pm$ 0.2	26.3 $\pm$ 0.1	24.0 $\pm$ 0.1	31.2 $\pm$ 0.1	28.3 $\pm$ 0.1	31.3 $\pm$ 0.1	-	30.0 $\pm$ 0.2	37.4 $\pm$ 0.2	30.2 $\pm$ 0.2
<i>Pfgl</i>	10.9 $\pm$ 0.3	19.5 $\pm$ 0.2	15.3 $\pm$ 0.2	17.5 $\pm$ 0.2	13.3 $\pm$ 0.3	20.1 $\pm$ 0.4	12.8 $\pm$ 0.2	19.1 $\pm$ 0.2	-	15.1 $\pm$ 0.1	14.5 $\pm$ 0.2	25.3 $\pm$ 0.2
<i>bulk</i>	20.7 $\pm$ 0.1	22.4 $\pm$ 0.1	25.4 $\pm$ 0.0	32.2 $\pm$ 0.1	24.6 $\pm$ 0.1	24.3 $\pm$ 0.1	31.8 $\pm$ 0.1	25.8 $\pm$ 0.1	-	33.2 $\pm$ 0.2	36.1 $\pm$ 0.2	31 $\pm$ 0.1
<i>Cfgl</i>	6.4 $\pm$ 0.5	15.9 $\pm$ 0.4	16.5 $\pm$ 0.1	19.0 $\pm$ 0.1	14.2 $\pm$ 0.4	10.2 $\pm$ 0.2	20.7 $\pm$ 0.2	17.0 $\pm$ 0.2	-	22.2 $\pm$ 0.2	21.9 $\pm$ 0.3	20.6 $\pm$ 0.2
<i>cgl</i>	-	32.1 $\pm$ 0.2			-	-	-	-	-	54.7 $\pm$ 0.2	44.1 $\pm$ 0.3	-

B.6 PYTHON SCRIPT FOR POROSITY MEASUREMENTS

Listing B.1: Python script for porosity measurements

```

#!/usr/bin/python
# -*- coding: utf8 -*-

import cv2
import numpy as np
from matplotlib import pyplot as plt
import random
import scipy
from scipy import ndimage
import matplotlib.patches as patches
import matplotlib.widgets as widgets

#Global variables to use in all functions
global xmin
global xmax
global ymin
global ymax

#Mouse on select
def onselect(eclick, erelease):
    global xmin
    global xmax
    global ymin
    global ymax
    print ' startposition : (%f, %f)' % (eclick.xdata, eclick.ydata)
    print ' endposition   : (%f, %f)' % (erelease.xdata, erelease.ydata)
    print ' used button    : ', eclick.button
    xmin = int(eclick.xdata)
    ymin = int(eclick.ydata)
    xmax = int(erelease.xdata)
    ymax = int(erelease.ydata)
    plt.close()

#Function definition for the running mean calculation
def runningMeanFast(x, N):
    return np.convolve(x, np.ones((N,))/N)[(N-1):]

#Read the three class file after QGIS processing
filename = '3class.tif'
wind = 15
img = cv2.imread(filename)

img = cv2.cvtColor(img, cv2.COLOR_BGR2GRAY)

h,w = img.shape
print "height=",h
print "width=", w
plt.figure(figsize=(20,10))
ax = plt.subplot(1,1,1)
plt.imshow(img,cmap='gray')

#Grab the user selection to use as the limits
rs=widgets.RectangleSelector(
    ax, onselect, drawtype='box',
    rectprops = dict(facecolor='red', edgecolor = 'black', alpha=0.5, fill=True))
plt.show()

```

```

print "Xmin=", xmin
print "Xmax=", xmax
print "Ymin=", ymin
print "Ymax=", ymax

fig, ax = plt.subplots(1)

plt.imshow(img, cmap='gray')
rect = patches.Rectangle((xmin, ymin), xmax-xmin, ymax-ymin, linewidth=3, edgecolor='r',
                        facecolor='none')
ax.add_patch(rect)

num_white = np.zeros(h)
num_black = np.zeros(h)
num_gray = np.zeros(h)
proc_gray = np.zeros(h)
norm_black = np.zeros(h)

for i in range(ymin, ymax):
    for j in range(xmin, xmax):
        if img[i, j] == 0:
            num_black[i] = num_black[i] + 1
        elif img[i, j] == 255:
            num_white[i] = num_white[i] + 1
        else:
            num_gray[i] = num_gray[i] + 1
    sum_gray = 0
    sum_white = 0
    sum_black = 0

for i in range(ymin, ymax):
    norm_black[i] = num_black[i] / (num_black[i] + num_white[i] + num_gray[i]) * 100
    proc_gray[i] = num_gray[i] / (num_white[i] + num_black[i] + num_gray[i])
    sum_gray = sum_gray + num_gray[i]
    sum_black = sum_black + num_black[i]
    sum_white = sum_white + num_white[i]

sum_total = sum_white + sum_black + sum_gray

#proc_gray = sum_gray / (sum_white + sum_black + sum_gray)
proc_white = sum_white / (sum_white + sum_black)
proc_black = sum_black / (sum_white + sum_black)

#print "The image contains ", proc_gray*100, "% of gray pixels"
print "White is ", proc_white*100, "% of black and white pixels"
print "Black is ", proc_black*100, "% of black and white pixels"

print "Total sum is ", sum_total
print "White sum is ", sum_white
print "Black sum is ", sum_black

ratio_bw = proc_black
error = 0.0

error = proc_gray * ratio_bw

print("Final error is %f", error)

#Calculation of the minimum pores + error
norm_black_error = norm_black + error*100

raw_norm_black = norm_black

norm_black = runningMeanFast(norm_black, wind)
norm_black_error = runningMeanFast(norm_black_error, wind)

```



```

for i in range(0,ymin):
    norm_black[i] = 0
    norm_black_error[i] = 0
for i in range(ymax,h):
    norm_black[i] = 0
    norm_black_error[i] = 0

file = open("porosity_data.csv","w")
file.write("height ; gray/total ; raw ; mov av ; mov av plus er ; error\n")
for i in range (0, h):
    file.write(str(i)+ " ; " + str(proc_gray[i]) + " ; " + str(raw_norm_black[i]) + " ; "
              + str(norm_black[i]) + " ; " + str(norm_black_error[i]) + " ; " + str(error[i]) +
              "\n")
file.close()

fig,ax = plt.subplots(1)

plt.ylim(0,h)
plt.xlim(0,100)

#Invert the axis y and put the x tick labels on the top
ax.xaxis.tick_top()
plt.gca().invert_yaxis()

#Plotting moving average with window wind
ax.axhline(y=ymin,xmin=0,xmax=100,c="blue",linewidth=2,zorder=0)
ax.axhline(y=ymax,xmin=0,xmax=100,c="blue",linewidth=2,zorder=0)
plt.plot(norm_black[ymin+wind:ymax-wind],range(ymin+wind,ymax-wind),label="Porosity",
         color='red')
plt.plot(norm_black_error[ymin+wind:ymax-wind],range(ymin+wind,ymax-wind),label="Porosity
         + error",linestyle='--', color='black')
plt.legend(frameon=False, fontsize=10,loc='best')

plt.show()

```


SUPPLEMENTARY FOR CHAPTER 4

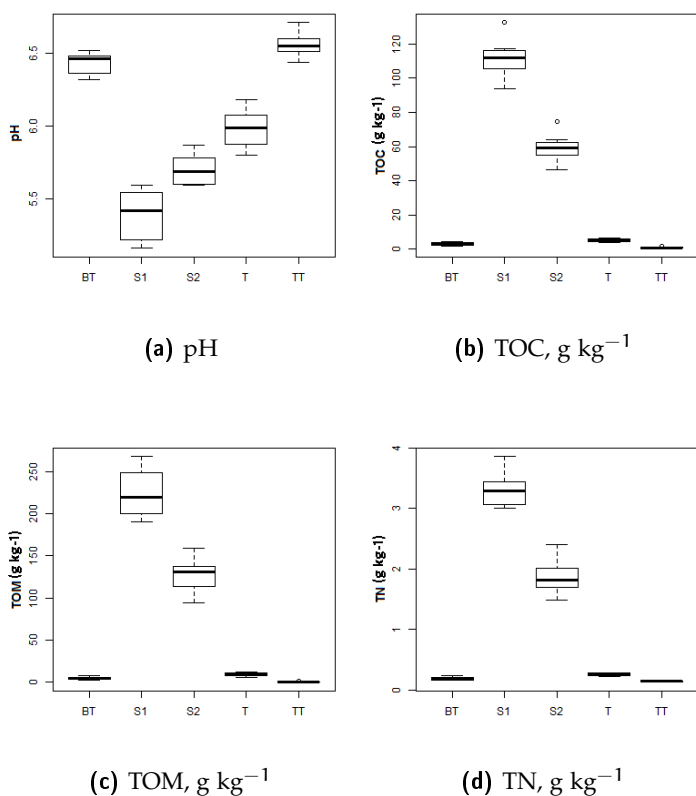
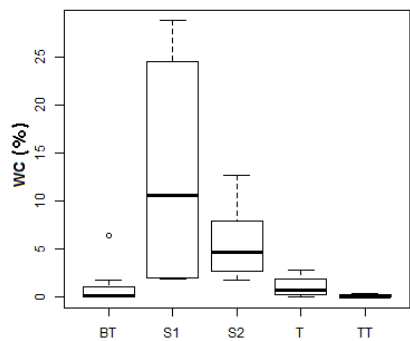
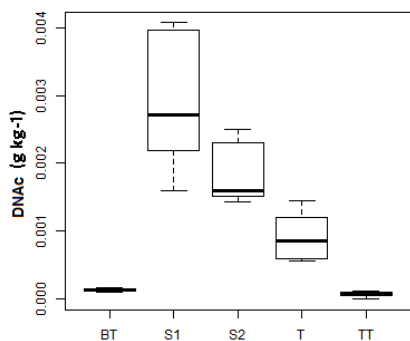


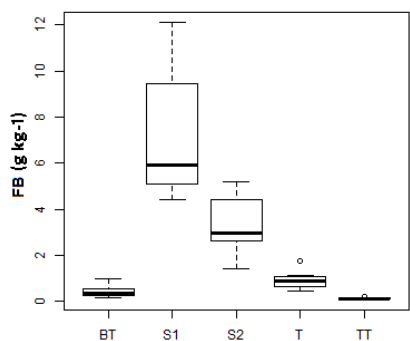
Figure C.1: Box plots showing differences between mean values obtained for TT, BT, T, S1 and S2 in both years (Pair-wise comparisons using Wilcoxon rank sum test, $p < 0.05$).



(a) WC, %



(b) DNA, g kg⁻¹



(c) FB, g kg⁻¹

Figure C.2: Box plots showing differences between mean values obtained for TT, BT, T, S1 and S2 in both years (Pair-wise comparisons using Wilcoxon rank sum test, $p < 0.05$).

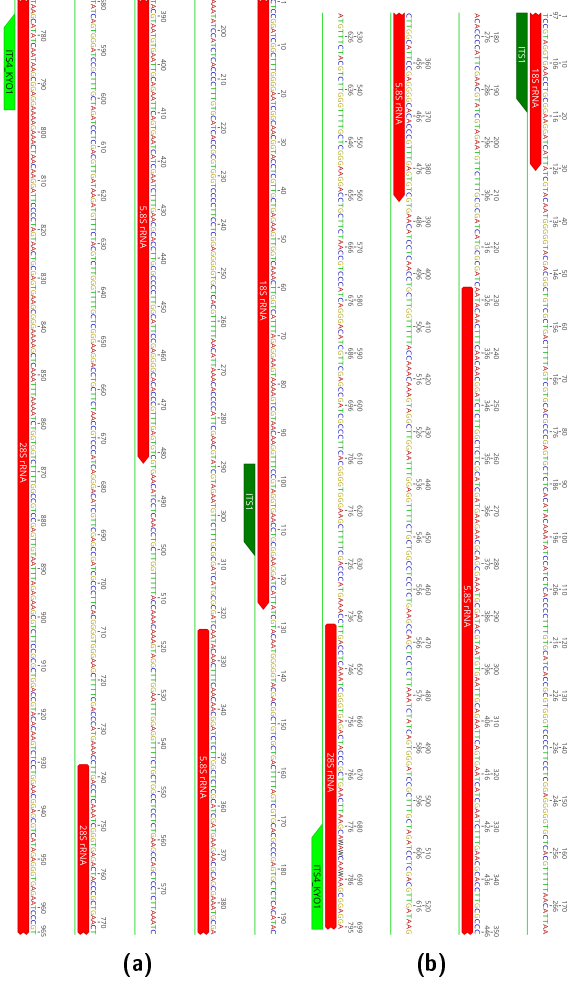


Figure C.3: In silico PCR amplification with hybridization of the primer pair ITS1 and ITS4-KY01 (a). PCR amplification size obtained with the in silico PCR amplification of *Russula brevipes* sequence (Accession number: FJ845429) using the primer pair ITS1 and ITS4-KY01 (b).

SUPPLEMENTARY FOR CHAPTER 5

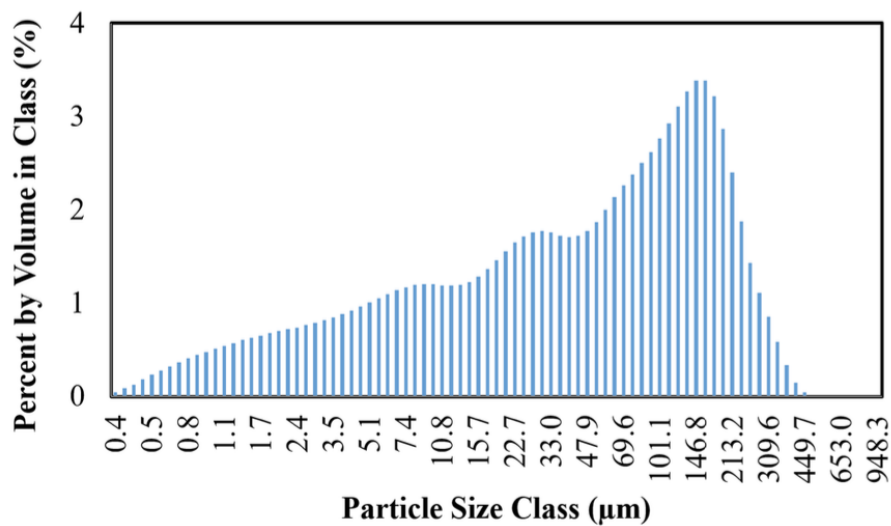


Figure D.1: Particle size distributions of Pinatubo ash samples sieved through 250 μm mesh, as measured using COULTER LS 100Q laser diffractometer.

Table D.1: Results from the two-way ANOVA. The effects on leaf temperature, plant-related and physiological parameters from treatment (controls, S1 and S2), days of measurements (or plant age) and the interaction between treatment and plant age. ns – nonsignificant. The data for chlorophyll fluorescence was excluded from the analysis, since the results were presented only for 2 days of measurements.

Parameters	p-values for different sources of variance		
	Plant age	Treatment	Age x Treatment
<i>H</i>	<0.001	<0.001	<0.001
<i>L</i>	<0.001	0.002	ns
<i>DW_s</i>	<0.001	<0.001	0.012
DW _r	<0.001	<0.001	<0.001
<i>WC_s</i>	<0.001	<0.001	<0.001
WC _r	<0.001	0.03	0.003
<i>A</i>	<0.001	<0.001	ns
<i>E</i>	<0.001	0.049	0.002
<i>g_s</i>	<0.001	0.033	<0.001
<i>WUE</i>	<0.001	<0.001	0.007
<i>c_i</i>	<0.001	0.045	<0.001
<i>T</i>	<0.001	<0.001	<0.001

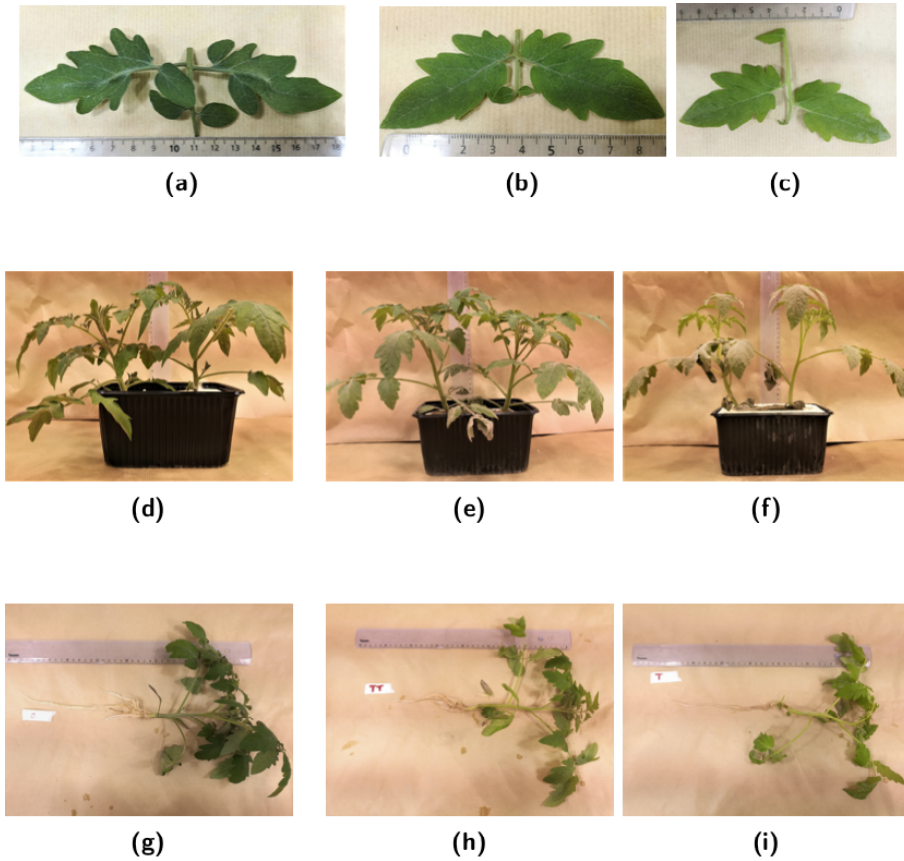


Figure D.2: Photographs of leaves of control tomato plants (a); tomato plants exposed to S1 after 14 days of experiment (b); exposed to S2 after 14 days of experiment (c); controls not treated with ash (d); plants exposed to S1 (e); plants exposed to S2 (f), aerial and root parts of controls (g); aerial and root parts of plants exposed to S1 (h); aerial and root parts of plants exposed to S2 (i).

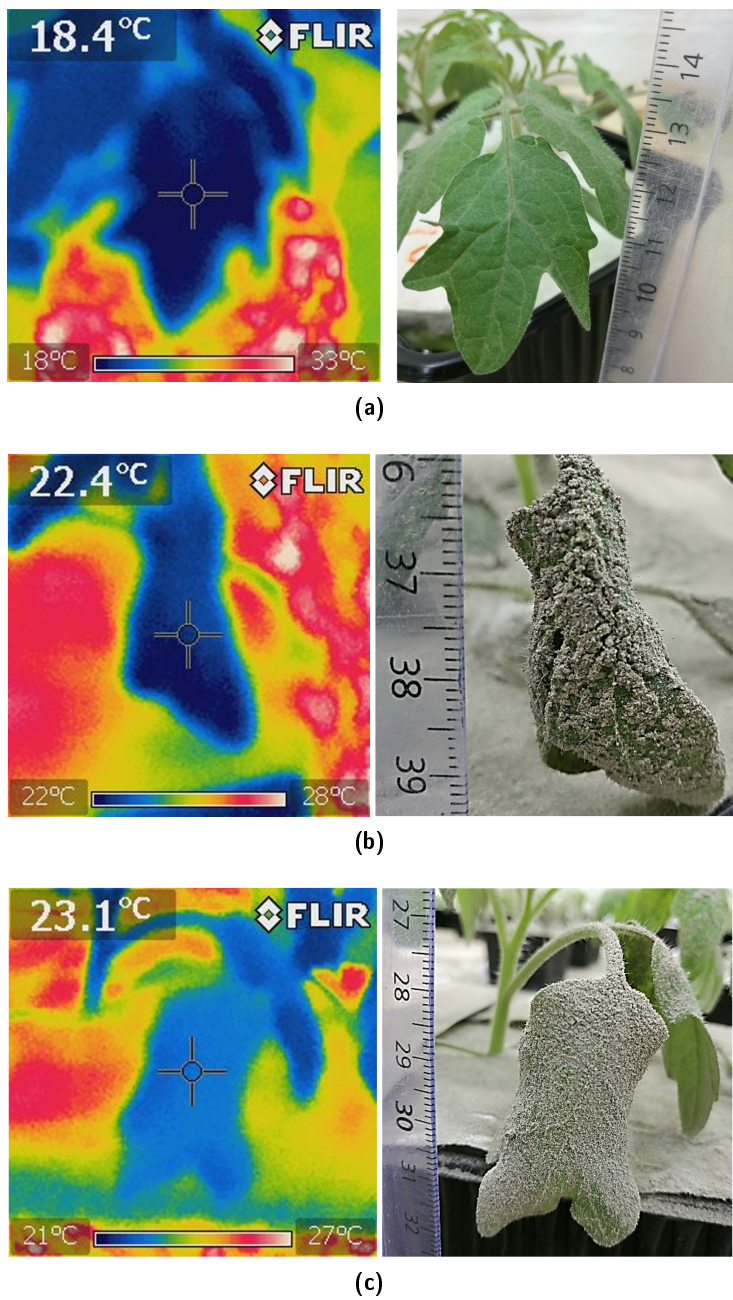


Figure D.3: Images obtained by IR-camera and photographs of the control leaf (a); S1 leaf (b) and S2 leaf (c).

SUPPLEMENTARY FOR CHAPTER 6

Table E.1: Results from the two-way ANOVA. The effects on leaf temperature, plant-related and physiological parameters from treatment (controls, S1 and S2), days of measurements (or plant age) and the interaction between treatment and plant age. ns – nonsignificant. The data for chlorophyll fluorescence was excluded from the analysis, since the results were presented only for 2 days of measurements.

Parameters	p-values for different sources of variance		
	Plant age	Treatment	Age x Treatment
H	<0.001	<0.001	0.002
L	0.023	0.331/ns	0.226/ns
DW_s	<0.001	<0.001	0.01
DW_r	<0.001	<0.001	0.014
WC_s	<0.001	<0.001	<0.001
WC_r	<0.001	<0.001	0.098/ns
A	<0.001	<0.001	0.006
E	<0.001	<0.001	<0.001
g_s	<0.001	0.24/ns	<0.001
WUE	<0.001	<0.001	<0.001
c_i	<0.001	<0.001	0.001
T	<0.001	<0.001	<0.001

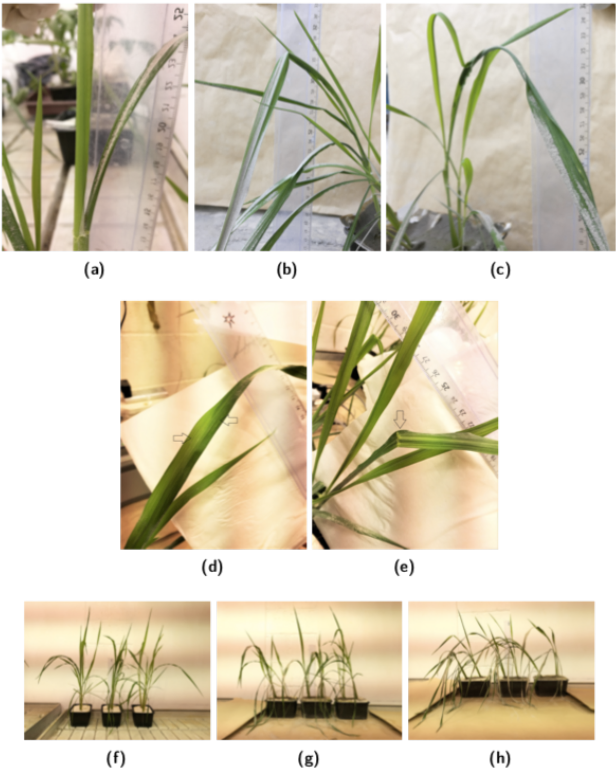


Figure E.1: Photographs of rice exposed to S2 (a); folded leaf treated with ash after 14 days of experiment (b); twisted leaf treated with ash after 14 days of experiment (c); leaf treated with ash after 14 days of experiment (with different shades of green on the twisted part of the leaf - showed on the image with black arrow) (d); folded leaf after ash removing after 14 days of experiment (arrow shows the different shades of green colour on the folded part) (e); control rice (f); rice exposed to S1 (g); rice exposed to S2 (h).

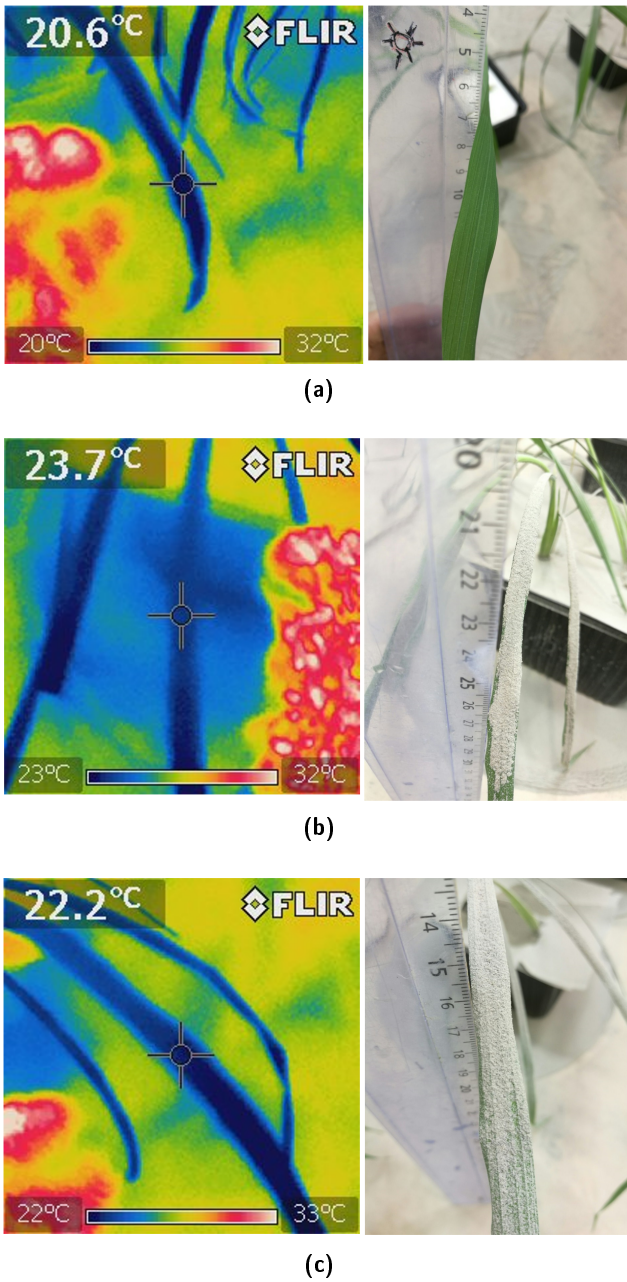


Figure E.2: Images obtained by IR-camera and photographs of the control leaf (a); S1 leaf (b) and S2 leaf (c).

CONFERENCES AND MISSIONS ABROAD

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F.2 MISSIONS ABROAD AND CONTRIBUTIONS TO OTHER PROJECTS

2014

- Fieldwork in Italy, Sicily, Mt Etna: 01/09/2014 - 16/09/2014.

2015

- Fieldwork in Italy, Sicily, Mt Etna: 07/09/2015 - 12/09/2015.
- Research stay for using the electron microprobe at the Ludwig-Maximilians- Universität (LMU), Department for Earth and Environmental Science, Germany; Munich: 14/12/2015 - 18/12/2015.

- Research stay for carrying out research related to volcanic ash abrasion at the Aarhus Universitet, Departament of Physics and Astronomy, Denmark, Aarhus: 01/06/2015 - 05/06/2016.

2016

- Secondment for conducting a field survey and scanning electron microscopy analyses in Ecuador, Quito: 08/10/2016 - 03/12/2016.

2017

- Fieldwork in Nicaragua, Mt. Masaya: training on volcanic soils affected by heavy acidic gas deposition from Masaya volcano. Helping two Msc projects associated to this initiative: (i) volcanic soil acidification in response to long-term volcanic acid deposition and (ii) assessment of heavy metal deposition fluxes downwind of a passively degassing volcano using a portable XRF: 11/02/2017 - 21/02/2017.

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