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ORIGINAL ARTICLE

Fungal diversity in the rhizosphere of endemic plant species of Tenerife (Canary Islands): relationship to vegetation zones and environmental factors

Christin Zachow[1], Christian Berg[2], Henry Müller[1], Remo Meincke[1], Monika Komon-Zelazowska[3], Irina S Druzhinina[3], Christian P Kubicek[3] and Gabriele Berg[1]
[1]*Institute of Environmental Biotechnology, Graz University of Technology, Graz, Austria;* [2]*Institute of Plant Sciences, Karl-Franzens-University of Graz, Graz, Austria and* [3]*Institute of Chemical Engineering, Research Area Gene Technology and Applied Biochemistry, Vienna University of Technology, Vienna, Austria*

Knowledge about fungal diversity scaling relationships relative to that of plants is important to understand ecosystem functioning. Tenerife Island, a natural laboratory to study terrestrial biodiversity, is represented by six different vegetation zones characterized by specific abiotic conditions and plant communities with a high proportion of endemic plants. Little is known about the biodiversity of associated fungi. To understand the relationship between plant and fungal communities, we analysed soil/rhizosphere fungi from all vegetation zones. From 12 sampling points dispersed on the whole island, molecular analysis of fungal communities was determined by single-strand conformation polymorphism (SSCP) analysis using universal and specific primers for *Trichoderma*. The highly diverse fungal communities were mainly characterized by ectomycorrhiza-forming Basidiomycota and a high proportion of yet-unidentified species. Besides, *Trichoderma*-specific SSCP resulted in low diversity of mainly cosmopolitan species, for example *Hypocrea lixii/T. harzianum.* The dominance of *T. harzianum* was confirmed by cultivation. All *Trichoderma* isolates show an extraordinarily high antagonistic potential towards different groups of plant pathogens, supporting the hypothesis of extensive colonization by highly competitive *Trichoderma* species from the continent. In contrast, biodiversity patterns of the whole fungal and plant communities follow the same ecological rules. Furthermore, a high statistical correlation between fungal communities and the main environmental factors, temperature and precipitation, was found.
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Macaronesia (Canary Islands, Madeira, Azores and Cape Verde Islands) has been recognized for a long time as a natural laboratory for model studies of diversification and development of terrestrial biodiversity (Emerson, 2002). In this study, we focused on Tenerife Island, with 2058 km^2, the largest of the Canary archipelago. Its altitudinal profile is remarkable: the island has a triangle-based pyramid circumference with a truncated apex at 2000 m a.s.l. at Las Caňadas and is surmounted by the summit of Teide (3718 m). The island is of vulcanic origin, with an estimated age of more than 7 million years. Tenerife has a well-investigated flora: the endemics represent approximately 40% of the native flora, though 20% are meanwhile in the endangered category of the International Union for Conservation of Nature (Francisco-Ortega *et al.*, 2000). Adaptive radiation into diverse habitats and genetic drift are often considered to be important factors producing such an extensive speciation (Baldwin *et al.,* 1998). On Tenerife Island, plants form six clearly differentiated vegetation belts of one plant community each, which were originally described by Humboldt and Bonplandt (1814). These vegetation zones strongly correlate with abiotic parameters such as temperature in summer and winter, altitude, wind and sun exposure, allocation of precipitation during the year, occurrence of fog and frost and soil quality. Although studies of animal and plant communities resulted recently in highly interesting results about evolution and diversity of species

Correspondence: G Berg, Institute of Environmental Biotechnology, Graz University of Technology, Petersgasse 12, Graz A-8010, Austria.
E-mail: Gabriele.Berg@TUGraz.at
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(Emerson, 2002; Green and Bohannan, 2006), nothing is known about the microflora of Tenerife Island and their relationship to vegetation zones and abiotic parameters.

Fungi play an important role in terrestrial ecosystems especially in the rhizosphere, where they mediate many ecological processes, and are influential for plant growth and soil health. The diversity of soil- and rhizosphere-inhabiting fungi is much higher than previously thought (Vandenkoornhuyse *et al.*, 2002; Gams, 2007). Molecular tools were developed to analyse the structure of terrestrial fungal communities (Kowalchuk, 1999; Vainio and Hantula, 2000; Anderson and Cairney, 2004; Berg *et al.*, 2005), but little is known about their diversity, biogeography and ecology, especially in native ecosystems. Furthermore, microorganisms comprise much of the Earth's biodiversity, but our knowledge about their biodiversity scaling relationships relative to that of plants and animals is limited (Green and Bohannan, 2006). There is a close link between plant species and microbial community structure in the rhizosphere, and the hypothesis is that bacteria and fungi have also developed a unique diversity pattern over time, which may give insights into microbial evolution.

The objective of this work was to analyse the associated fungal communities of six different vegetation zones in Tenerife. Microfungi were isolated from 12 different sampling points and analysed by a multiphasic approach to analyse the relationships between the plant community, the associated fungal communities and abiotic parameters. Particular interest was laid on the analysis of members of the genus *Trichoderma*, a fungal genus that is often a predominant component of soil microflora and plays a key role in soil health (Harman *et al.*, 2004; Berg *et al.*, 2005; Migheli *et al.*, 2008).

Materials and methods

Experimental design and ecological characterization of sampling points

Sampling was organized to cover all six main vegetation zones throughout Tenerife Island twice (Figure 1). Data about the vegetation zones and sampling points are shown in Table 1. To select a sampling point, we search for a typical appearance of vegetation and a high level of hemeroby within each vegetation zone. The second sampling point should be distant as much as possible from the first. The abiotic differences in the six vegetation zones are extraordinarily high within a comparable little territory. The main abiotic parameters such as allocation of temperature and precipitation during the year, altitude, exposure, fog and soil quality can easily be summarized in the two most important climatic factors, annual temperature and precipitation. These data were measured for a long time on Tenerife Islands; in Table 1, they are summarized from Huetz de Lemps (1969), Kämmer (1974) and Fernandopullé (1976). Furthermore, soil types on

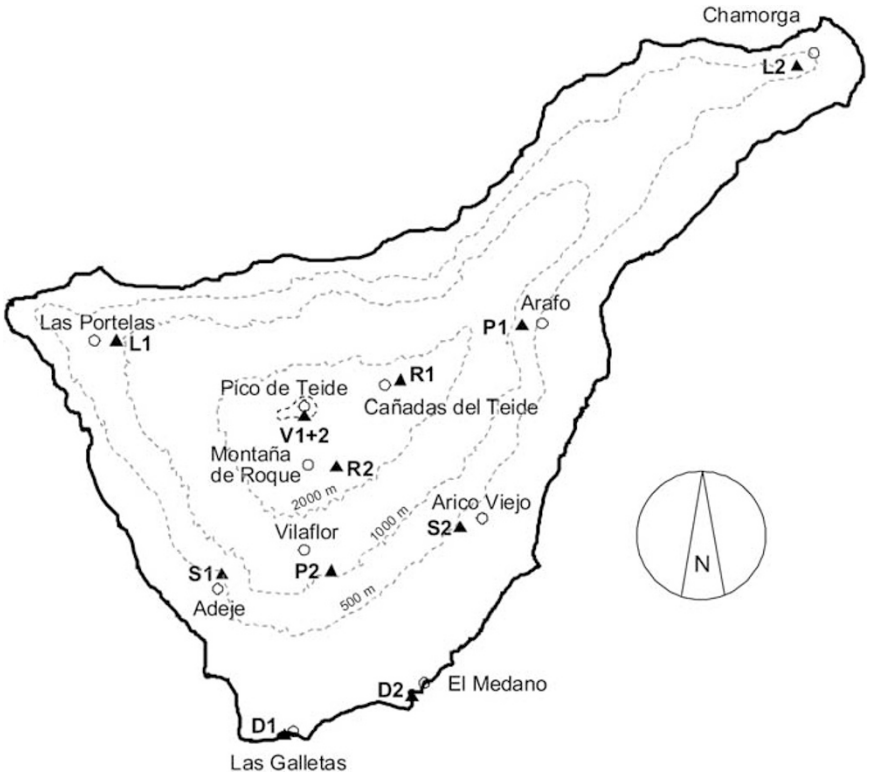

Figure 1 Tenerife Island map with some orientation settlements (circles) and location of sampling points (triangles).

Table 1 Location, altitude and vegetation of the sampling points, Tenerife Island, February 2007

Abb.	*Date*	*Location*	*Coordinates*	*Alt*[a]	*Sampling grid*		*Climatic characteristics*	
					Classification	*Vascular plants*[b]	°*C*[c]	*mm*[d]
D1	06-02-2007	1.5 km W Las Galletas	28°00′27″N 16°39′59″W	1 m	Desert and coastal dune vegetation: Zygophyllum-Zollikoferia semidesert (subtropical, arid)	*Argyranthemum frutescens*[e], *Launeaea arborescens*, *Fagonia cretica*, *Schizogyne serriata*[e]	23.5	100
D2	06-02-2007	1.0 km SW El Medano	28°02′22″N 16°32′38″W	2 m		*Atriplex glauca* var. Ifniensis, *Launeaea arborescens*, *Lotus sessilifolius*[e], *Polycarpaea nivea*, *Schizogyne serriata*[e]		
S1	06-02-2007	1.2 km NE Adeje	28°07′53″N 16°43′08″W	480 m	Succulent zone (Cardonal/Tanaibal): *Kleinio nerifolii-Euphorbietea canariensis* (subtropical, arid)	*Asphodelus aesitivus*, *Euphorbia regis-jubae*[e], *Hyparrhenia hirta, Kleinia neriifolia*[e], *Lavendula canariensis*[e], *Opuntia dillenii*, *Plocama pendula*[e], *Taekolmia pinnata*[e]	18.5	250
S2	09-02-2007	2.5 km SW Arico Viejo	28°10′13″N 16°30′07″W	510 m		*Aeonium cf. urbicum*[e], *Anagallis arvensis*, *Asparagus pastorianus*[e], *Euphorbia regis-jubae*[e], *Hyparrhenia hirta*, *Kleinia neriifolia*[e], *Lavendula canariensis*[e], *Periploca laevigata, Taekolmia pinnata*[e]		
L1	10-02-2007	0.9 km E Las Portelas	28°19′43″N 16°49′59″W	950 m	Laurel wood (Laurisilva/Fayal-Brezal): *Pruno hixae-Lauretea azoricae* (subtropical, subhumid)	*Asplenium onopteris, Dryopteris oligodonta*[e], *Ilex canariensis*[e], *Laurus azoricus*[e], *Picconia excelsior*[e], *Ranunculus cortusifolius*[e]	12.5	650
L2	12-02-2007	1.5 km SW Chamorga	28°33′34″N 16°10′08″W	800 m		*Dryopteris oligodonta*[e], *Laurus azoricus*[e], *Prunus lusitanica*		
P1	09-02-2007	2.4 km W Arafo	28°20′24″N 16°26′36″W	990 m	Canary pine forest (Pinar): *Cytiso proliferi-Pinetea canariensis* (warm tempered, semiarid)	*Cistus monspeliensis, Cistus symphytifolius*[e], *Pinus canariensis*[e]	15.0	400
P2	09-02-2007	3.7 km SE Vilaflor	28°07′56″N 16°36′52″W	990 m		*Cistus monspeliensis Cistus symphytifolius*[e], *Pinus canariensis*[e]		
R1	08-02-2007	1.5 km NE Las Cañadas del Teide	28°18′07″N 16°33′49″W	2100 m	Mountainous heath land (Retamar-Codesar): Spartocytisetea (tempered, arid, subalpine)	*Descurainia bourgaeana*[e], *Pterocephalus lasiospermus*[e], *Spartocytisus supranubius*[e]	8.8	345
R2	08-02-2007	1 km E Montaña de Roque	28°13′20″N 16°37′10″W	2200 m		*Pterocephalus lasiospermus*[e], *Spartocytisus supranubius*[e]		
V1	13-02-2007	Pico de Teide	28°16′12″N 16°38′19″W	3480 m	Lava rock alpine desert (Violeta): *Violetea cheiranthifoliae* (tempered, arid, alpine)	No vascular plants	7.5	350
V2	13-02-2007	Pico de Teide	28°16′11″N 16°38′20″W	3480 m		No vascular plants		

[a]Alt = altitude above see level.
[b]Vegetation types and recorded vascular plants at the sampling points (nomenclature followed Hansen and Sunding (1985).
[c]Annual mean temperature in °C.
[d]Annual precipitation in mm.
[e]Endemic plant species in Macaronesia.

Tenerife Island are well studied. Different soil types have been produced due to the effects of vegetation, local weather conditions and topography on the basis of volcanic materials (Fernández Caldas *et al.*, 1987; Hernández-Moreno *et al.*, 2007). At the area of 1 m^2 of each sampling point, the composition of vascular plants was analysed and the altitudes above see level were measured.

Sampling

Soil/rhizosphere was sampled from each sampling point in February in 2007. At each sampling point, four independent samples were taken in the corners of a 1 m^2. We collected about 2 ml of soil from a depth of approximately 10 cm. Samples were placed into sterile Eppendorf tubes and transported to the laboratory.

Analysis of the community structure by single-strand conformation polymorphism analysis and identification of bands

Microorganisms of the soil communities were extracted by mechanical disruption and homogenization of 400 μg soil in a FastPrep Instrument (Qbiogene, BIO101 Systems, Carlsbad, CA, USA) for 30 s at a speed 5.0 m s^{-1}. DNA was purified by the GeneClean Turbo Kit (Qbiogene, BIO101 Systems) containing the special binding buffer guanidine thiocyanate for the removal of humic acids. Extracted DNA (8 μl) was treated with RNase (2 μl, 0.1 mg ml^{-1}) for 5 min at 65 °C to get the template for the first PCR applications. The procedure of single-strand conformation polymorphism analysis (SSCP) was performed according to Schwieger and Tebbe (1998). Fungal and *Trichoderma* communities were analysed using the internal transcribed spacer region (ITS) primer pair ITS1f/ITS4r (White *et al.*, 1990) in a first reaction mixture of 20 μl PCR Mastermix (Tag&Go, Qbiogene), 0.5 μM each primer, 2.5 mM $MgCl_2$ and about 20 ng of template. *Trichoderma* communities were analysed using the *Trichoderma* primers uTf/uTrP (Hagn *et al.*, 2007) in a first reaction mixture of 20 μl PCR mix (DyNAzym EXT DNA Polymerase Kit; New England Biolabs, Ipswich, MA, USA) containing 10 pmol of each primer, 0.2 mM dNTPs each, 1 × reaction buffer, 2.5 mM magnesium chloride and 2.5 U DyNAzyme Polymerase with the addition of 0.3% bovine serum albumin and 5% dimethyl sulphoxide (Sigma Aldrich, Steinheim, Germany) and about 20 ng template. To obtain genetic fingerprints of fungal as well as *Trichoderma* communities, a nested PCR was performed. In the second PCR, the fungal-specific primer pair ITS1f/ITS2rP (White *et al.*, 1990) and the *Trichoderma*-specific primer pair uTf/ITS2P, respectively, were used in a 60 μl reaction mixture containing 12 μl PCR Mastermix (Taq&Go; Qbiogene, BIO101), 0.5 μM of each primer, 2.5 mM $MgCl_2$ and 10 μl of PCR product from the first PCR as template. The PCR products were purified by the GeneClean Turbo Kit (Qbiogene, BIO101), before a λ-exonuclease (New England Biolabs) digestion and DNA single-strand folding according to Lieber *et al.* (2003). The polyacrylamide gel electrophoresis was performed on a TGGE apparatus (Biometra, Göttingen, Germany) at 26 °C and 400 V for 19 h using 9% (wt vol^{-1}) acrylamide gels. Afterwards, the gels were silver-stained according to the procedure of Bassam *et al.* (1991).

Conspicuous DNA bands of the community patterns were eluted from the gel by suspending the gel slice in 150 μl elution buffer, containing 0.5 M ammonium acetate (Sigma, Vienna, Austria), 10 mM magnesium acetate tetrahydrate (Sigma), 1 mM EDTA (pH 8.0, AppliChem, Darmstadt, Germany) and 0.1% (wt vol^{-1}) SDS (AppliChem), for 3 days at 4 °C, following DNA ethanol precipitation, centrifugation and resuspension in 10 mM Tris-HCl (pH 8.0). Gel-extracted DNA was reamplified by using primer ITS1/ITS2P for fungi and uTf/ITS2P for *Trichoderma*. Fragments of the expected size were sequenced with the Applied Biosystems (Foster City, CA, USA) 3130 × l Genetic Analyser sequencer Data Collection v. 3.0, Sequencing Analysis v. 5. For phylogenetic analysis and identification of sequences from SSCP bands, the BLAST algorithm according to Altschul *et al.* (1997) was used. To this end, sequences were first manually inspected for the presence of signature shifts indicative of the presence of chimaeric sequences and also tested for their presence by the alignment to Bellerophon (Huber *et al.*, 2004), and only sequences devoid of chimaeras were used for the BLAST search.

Computer-assisted cluster analysis and conformational grouping (OTUs)

Computer-assisted evaluation of fungal and *Trichoderma* community profiles obtained by SSCP was performed by using the GelCompar software (Applied Math, Kortrijk, Belgium). The silver-stained SSCP gels were scanned using a transmitted light scanner (Epson perfection 4990 Photo, Nagano, Japan) to obtain digitized gel images. The gels were normalized, which allowed band pattern of different gels to be compared, and the background was subtracted (details at the manufacturer's manual). The Pearson's correlation index (r) for each pair of lanes within a gel was calculated as a measure of similarity between the community fingerprints. Finally, cluster analysis was performed by applying the unweighted pair-group method using average linkages to the matrix of similarities obtained.

DNA fragments of same length but different base composition can be separated based on their three-dimensional conformation. SSCP is based on the differences in the conformation of single-stranded DNA fragments. The electrophoretic mobility of the single-stranded DNA fragments depends on their three-dimensional conformation. Each of the amplifi-

cation products was identified by its electrophoretic distance on SSCP gel and the number of DNA fragments. According to the distance of the bands, the SSCP gels were theoretically divided into 40 operational taxonomic units (OTUs). The presence or absence of individual amplified product DNA bands in each group was scored. The obtained matrix was used to compare data for correspondence analysis (see statistics).

Quantitative PCR

Quantitative analyses were performed according to Hagn *et al*. (2007) with some modifications. Briefly, plasmid standards for the quantification of environmental *Trichoderma* spp. DNA were prepared from a cloned *Trichoderma reesei* (EF070658; Grosch *et al.*, 2006) ITS1/2 fragment ligated into a pGEMT Easy Vector System (Promega, Madison, WI, USA). Amplification of extracted DNA from soil as well as from standards was carried out with an ABI 3700 Real Time PCR System. Reaction components per 20 µl included 1 µl of DNA, 5 µl qPCR Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CI, USA) and 3 pmol of each primer. The cycler conditions were as follows: initial 95 °C for 10 min followed by 30 cycles of 95 °C for 30 s, 60 °C for 1 min and detection. Each standard, control and sample was prepared in triplicates and in serial dilutions for the soil. Cycling, data collection and calculation were performed by the ABI 3700 Software according to the manufacturer's instructions. After quantification, the samples were analysed by melting curve calculation and agarose gel electrophoresis for quality control of the PCR.

Isolation and characterization of fungi and Trichoderma

The soil dilution plate method was used for isolation of *Trichoderma.* Approximately 100 µg of soil was mixed with 0.85% sterile NaCl and spread on 90-mm diameter petri plates containing *Trichoderma* selective medium (TSM; Smith *et al.,* 1990), on potato dextrose agar (PDA; Merck & Co., Whitehouse Station, NJ, USA) amended with 100 µg ml^{-1} streptomycin and tetracycline, respectively, and on TSMC (Elad and Chet, 1983). Plates were incubated at 25 °C with daily light conditions. Two plates per suspension were prepared, giving six plates per substrate (TSM, TSMC or PDA). After 7 days, colony-forming fungi were counted to calculate the means of colonies (log_{10} colony-forming unit (CFU)) based on fresh weight, and all colonies resembling *Trichoderma* were transferred to plates containing PDA and incubated as above. *Trichoderma* colonies were subsequentially subcultured using single-spore technique and stored at 4 °C until DNA extraction. All isolates described in this study are conserved as plugs in liquid storage medium containing glycerol (final concentration 15%) in the strain collection SCAM (strain collection for antagonistic microorganisms) at the Graz University of Technology (Austria).

All strains were characterized by their BOX fingerprint (Rademaker and De Bruijn, 1997) using the BOXA1R primer (5′-CTACGGCAAGGCGACGCT GACG-3′). The variability of BOX-PCR patterns was assessed by fingerprinting five strains in three independent experiments. Computer-assisted evaluation of *Trichoderma* community profiles obtained by BOX-PCR was performed by using the GelCompar program. Cluster analysis was performed with the unweighted pair-group method with arithmetic average algorithm.

Species identification

To identify *Trichoderma* isolates at the species level, a fragment of the nuclear rRNA comprising ITS1 and -2 and the 5.8S rRNA gene were amplified and sequenced as described earlier (Druzhinina *et al.*, 2005). In addition, the fourth large intron of the translation elongation factor 1-α (*tef1*) gene was also amplified if necessary as described by Druzhinina *et al*. (2004). Automated sequencing was performed with an ABI Prism 3100 DNA Sequencer (Applied Biosystems, Norwalk, CT, USA) at the sequencing core facility CRIBI—Bio Molecular Research at the University of Padova, Italy. ITS1 and -2 sequences were subjected to analysis by *TrichO*KEY (http://www.isth.info/tools/molkey/index.php; Druzhinina *et al.*, 2005). In ambiguous cases, usually common for section *Trichoderma*, the result was re-checked by analysis of the large intron of *tef1* gene sequence using sequence similarity search against a database of type sequences implemented in TrichoBLAST (www.isth.info/tools/blast; Kopchinskiy *et al.*, 2005). For analysis of unusual ITS1 and -2 or *tef1* alleles, sequences were automatically aligned with GeneDoc 2.6.002, manually edited and inspected by eye. Potentially unique alleles were then confirmed by sequence similarity search against NCBI GenBank and a database of fungal strains of Vienna University of Technology that currently contains more than 2700 *Hypocrea/Trichoderma* strains with more than 3300 sequences. A haplotype was considered to be unique if at least one allele (ITS1 and -2 or *tef1*) did not occur in any other strain isolated outside of Tenerife.

Screening selected Trichoderma *isolates for antagonistic potential to plant pathogens*

The *in vitro* inhibition of *Botrytis cinerea* Pers.*, Guignardia bidwellii* (Ellis) Viala & Ravaz, *Rhizoctonia solani* Kühn AG2-2IIIB and AG4, *Sclerotium rolfsii* Sacc., and *Verticillium dahliae* Kleb. V25 were determined by a dual-culture assay on PDA or Waksman agar according to Berg *et al*. (2002). Zones of hyperparasitism were measured after 3–7 days of incubation at nearly 25 °C.

Statistics
Correspondence analysis was used to answer the question whether a correlation exists (1) between the independently sampled fungal communities of the different sampling points and (2) between fungal communities and environmental data. For this, we used the indirect correspondence analysis (CA) and direct correspondence analysis for unimodal data of the software package Canoco 4.5 (Lepš and Šmilauer, 2003).

Nucleotide sequence accession numbers
Sequence accession numbers for sequences submitted to the NCBI nucleotide sequence database are for the fungal strains EU870043 to EU870093, and for the *Trichoderma* strains EU870094 to EU870135 and EU871008 to EU871036.

Results

Plant communities and habitat characterization
Owing to the extraordinarily significant differences in climate and altitude on Tenerife Island, the different plant communities under investigation are extremely distinct and correspond to main climatic and oreographic vegetation zones (Table 1). Each vegetation zone is presented by one main plant community that we analysed. Data obtained for the composition of vascular plant species were highly similar for the two different sampling points within one vegetation zone. On the other hand, vascular plant data showed clear differences between the plant communities of different zones. Two communities belong to the subtropical, arid vegetation. First, the open dune vegetation (D) resembling a semidesert with scarce occurrence of succulent shrubs like *Atriplex glauca* var. *ifniensis*, *Launeaea arborescens* and *Schizogyne serriata*. Second, the succulent zone (S) rich of endemics is typical for lower altitudes in South Macaronesia, in Tenerife characterized by *Euphorbia regis-jubae*, *Kleinia neriifolia*, *Lavendula canariensis, Plocama pendula* and *Taekolmia pinnata*, for instance. The conditions of two further zones are arid as well but plant communities belong to the more temperate zone due to their occurrence in higher altitudes: the subalpine mountainous heathland (R) with *Descurainia bourgaeana, Pterocephalus lasiospermus* and *Spartenocytisus supranubius* as typical vascular plants, and the alpine desert vegetation (V) around the Pico de Teide, where the endemic *Viola cheiranthifolia* is found but lacking in our data set because *V. cheiranthifolia* appears only during summer time. All these communities are open vegetation types without trees. The last two communities are woodland: the semiarid Canary pine forest (P) with *Pinus canariensis* and *Cistus* species, and the Laurel wood (L) with vascular plants like *Ilex canariensis, Laurus azoricus, Picconia excelsior* and *Dryopteris oligodonta*; the only vegetation type with humid conditions. Analysing our data by direct correspondence analysis, a high correlation between the plant communities and abiotic data (precipitation, temperature and altitude) was found (data not shown).

Fungal and Trichoderma *communities: molecular fingerprints*
To study the diversity of the whole fungal communities, all samples were characterized by the molecular fingerprint using ITS-PCR SSCP. For all samples, between 20 and 40 bands for the total fungal (Figures 2a and b) and between one and six bands for the *Trichoderma*-specific community (Supplementary Figure S1) were obtained. All patterns demonstrated a high diversity by expressing different amounts and intensities of fungal bands in all three replicates. However, the statistical analysis using GelCompar resulted in vegetation zone-specific groups (data not shown). For universal as well as group-specific community patterns, dominant and unique bands were detectable and sequenced for identification (see paragraph below). Furthermore, GelCompar bands were grouped and numbered into OTUs to analyse the diversity of fungal communities on molecular basis as well as their relationship to plant communities and environmental factors.

Correspondence analysis of fungal communities and environmental factors
Indirect correspondence analysis based on the OTUs of fungal species can show the coherence and similarity of the different samples indicated by crowding points (surrounded by ellipsoids) at a CA biplot. The analysis resulted in surprisingly clear patterns (Figure 3). All six samples (three replicates of two sampling points) of the same plant community clustered together in each case except for the desert and costal dune vegetation (D). The Laurel wood (L) is the most extraordinary and only humid ecosystem in the Macaronesian archipelago. Its fungal community showed the same unique pattern, correlated with precipitation at the second axis. These samples are the most different ones within the whole data set. The samples of semiarid and arid zones are arranged along the first axis. It is correlated with altitude as well as temperature, which itself is nearly a reciprocal value of altitude. Along this axis, the fungal communities are arranged on a gradient from high to low temperature: desert (D), pine forest (P), succulent zone (S), subalpine (R) and alpine vegetation (V); the types R and V right from the centre are characterized by an increasing occurrence of frost. According to this gradient of temperature, the situation of P between D and S is unexpected; it would be expected between S and R.

The direct gradient analysis of Figure 4 shows the relationship of the samples and the response of the

OTUs with regard to the environmental factors. The picture is clearer than in Figure 3; the P-characterizing OTUs are between S and R and have a correspondence to L, which is a woody habitat as well. In the temperature-determined 'semiarid line' D–S–P–R–V, the P units are positively and the D units are negatively influenced by precipitation, showing that these species are more adapted to moist or dry soil condition, respectively.

Identification of fungal species

Altogether, 53 fungal clones were cut out from SSCP gels (Figure 2), sequenced and compared with sequences deposited in GenBank. Dominant and unique bands from SSCP gels, and outstanding OTUs resulting from corresponding analysis (Figure 4) were selected. Surprisingly, with two exceptions, all sequences were designated to Basidiomycota (Table 2). Many sequences closely matched with sequences from mycorrhizal fungi or uncultured strains. However, the majority of isolates had a very low index of similarity to known species or other strains in the databank. There are several isolates that could be identified on genus level; they belong to *Cryptococcus, Hebeloma* and *Inocybe*. Species of the *Cryptococcus* genus occurred ubiquitously, mainly in the vegetation zone Laurisilva (L), mountain heathland (R) and on Pico de Teide (V). They were found especially in high altitudes; in R, for example *C. aerius* (ITS9, -37 and -41), *C. bhutanensis* (ITS36 and -40) and *C. phenolicus* (ITS35 and -38), and in V, for example *C. albidus* (ITS46 and -50), *C. antarcticus* (ITS52), *C. friedmannii* (ITS49) and *C. vishniacii* (ITS47). *C. podzolicus* (ITS137, OTU35) was found only in Laurel wood (L). According to the results of our correspondence analysis, the latter was found to be

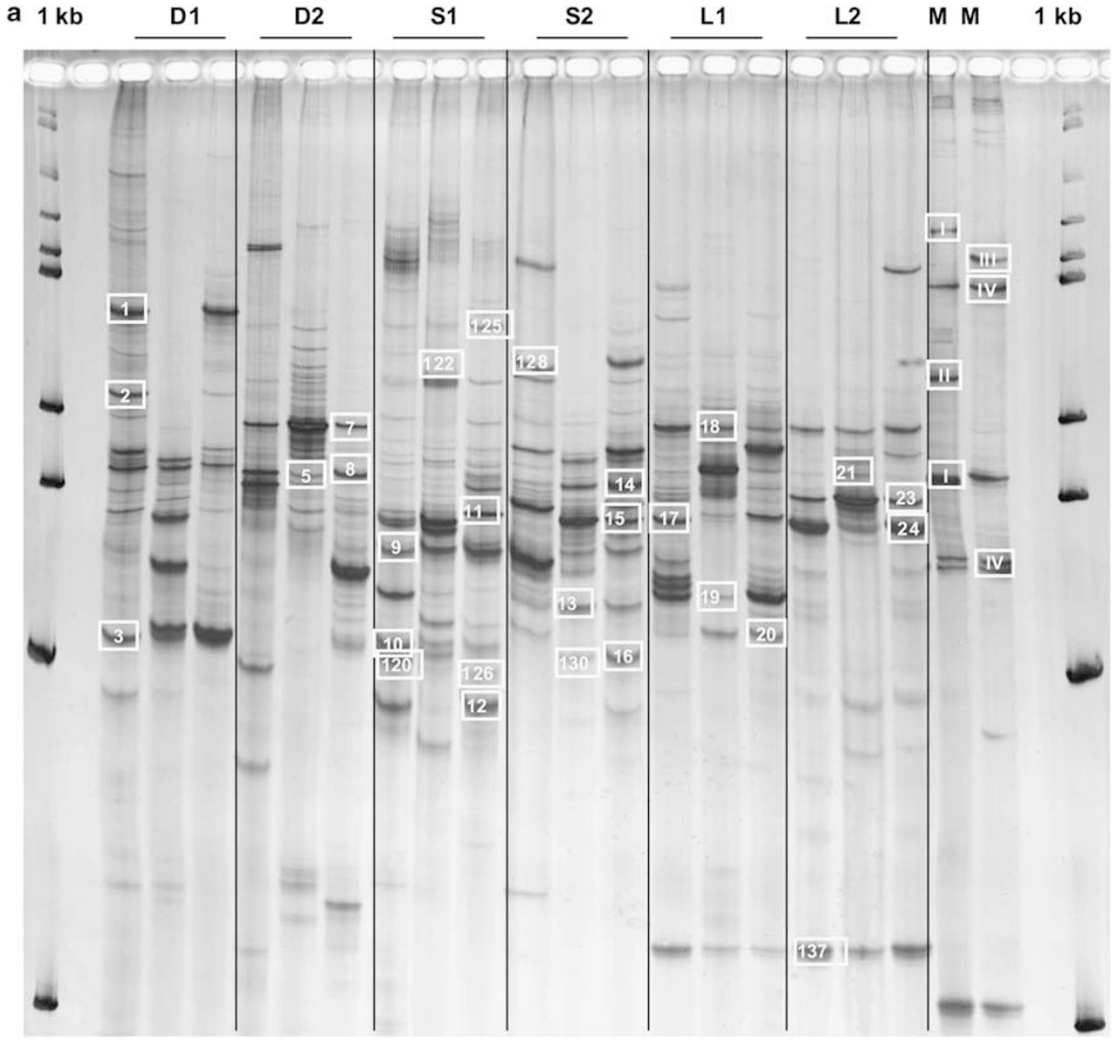

Figure 2 SSCP profiles showing the fungal communities from 13 different sampling locations in Tenerife (**a**) D1, D2, S1, S2, L1, L2 and (**b**) P1, P2, R1, R2, V1, V2 obtained using ITS1 and ITS4 primers. Lane marked M contains PCR products from pure fungal cultures: I = *Rhizoctonia solani* AG2-2IIIB, II = *Trichoderma gamsii* AT1-2-4, III = *Verticillium dahliae* V25, IV = *T. cerinum* G1/9 and *T. viride* G3/2, and V = *T. cerinum* G1/9. White framed bands were excised for identification by sequencing (for results see Table 4). ITS, internal transcribed spacer region; SSCP, single-strand conformation polymorphism.

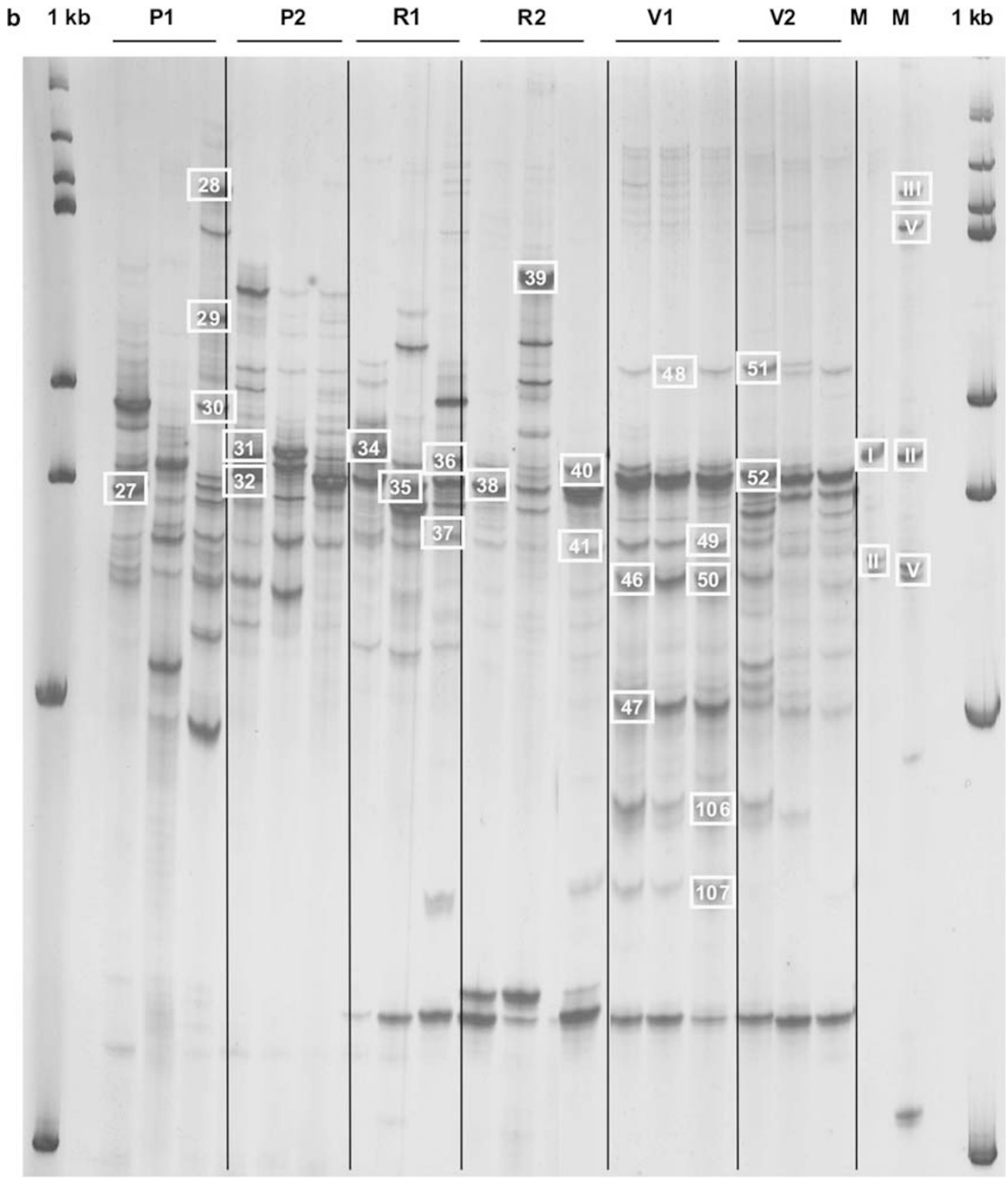

Figure 2 Continued.

specific for this vegetation zone. Other specific species were *Hebeloma cistophilum* (ITS29) in the Canary pine forest (P) and *Noahidea sebacea* (ITS107) on Pico de Teide (V). Other dominant bands were identified as *Rhizoctonia* spp. (ITS1, -3, -14 and -20), especially in the desert zone (D). In the succulent zone (S), mainly uncultured isolates were identified. In contrast, species of OTU groups 12, 13, 15 and 17 showed a ubiquitous behaviour.

In addition, 42 clones from the *Trichoderma* were sequenced (Table 3). Although the fragments allowed only the sequencing of a part of ITS1, and thus clear diagnostic sequences were not obtained, all but one clone were safely identified as *Trichoderma/Hypocrea*, section *Trichoderma* and section *Pachybasium*. The analysis revealed a low diversity of *Trichoderma* species. Yet because of hallmark areas within the sequences, three isolates could be identified at the species level (*T. stromaticum, T. spirale* and *H. cremea*). The majority of isolates (29) exhibited an allele indicative of either *T. harzianum* or *T. tomentosum*. One isolate (TR 13) exhibited a new allele with affinity to the *H. lixii/catoptron* clade. Five isolates belonged to section *Trichoderma* and could not be identified closer. Interestingly, according to correspondence

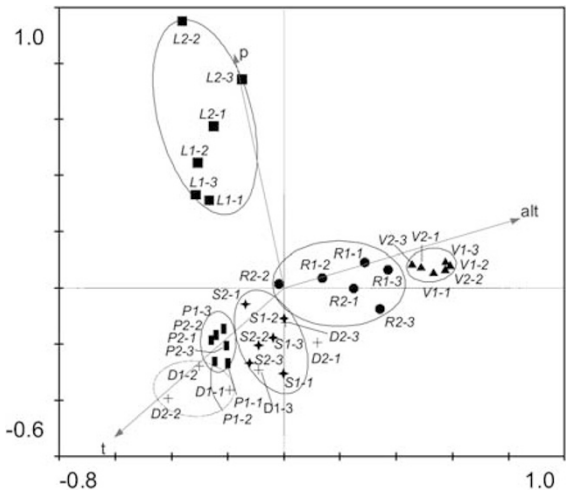

Figure 3 Correspondence analysis (indirect unimodal gradient analysis, interspecies distances, biplot scaling) of sampling sites based on operational taxonomic units of fungi community data. Eigenvalues of first and second axes are 0.24 and 0.21, respectively; sum of all eigenvalues is 1.63. The environmental data are *post hoc* given as Supplementary Data. Ellipsoids show the centre of the vegetation type. T = temperature, p = precipitation, alt = altitude.

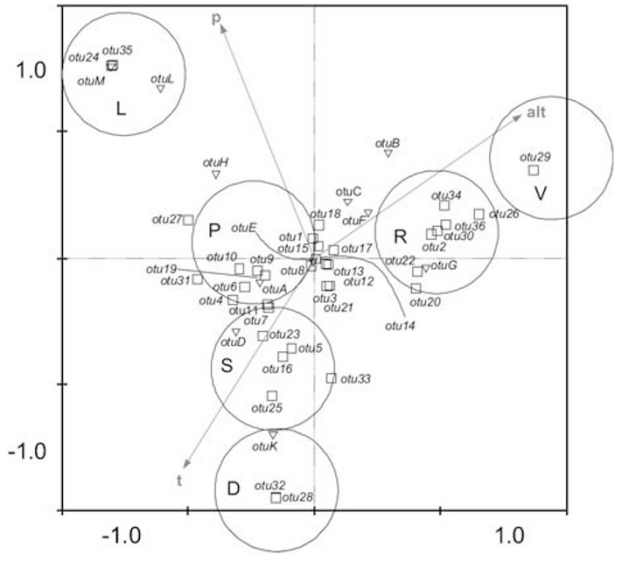

Figure 4 Canocial correspondence analysis triplot (direct unimodal gradient analysis) of operational taxonomic units (OTUs). Squares (labelled with figures) show the units from ITS data and triangles (labelled with letters) the units from *Trichoderma* data set. Circles indicate the location of the sampling sites. Eigenvalues of first and second axes are 0.21 and 0.17, respectively; sum of all eigenvalues is 1.63. Environmental data are predetermined. T = temperature, p = precipitation, alt = altitude. ITS, internal transcribed spacer region.

analysis, *T. viride* was found to be specific for the Laurel wood (L).

Fungal abundances, isolation and characterization of Trichoderma *strains*

From all sampling points, fungal as well as *Trichoderma* strains were isolated on different media. Altogether, CFU counts on synthetic low-nutrient agar (SNA) were in the range of $\log_{10}$ 3.95 and 6.97 CFU per g soil fresh weight (sfw) (average 5.41). The CFU numbers determined on PDA were slightly but not significantly higher and ranged from $\log_{10}$ 4.62 to 7.04 CFU per g sfw (average 6.55). Although no statistically significant differences were observed between the sampling points, especially in the desert zone (D) and in Teide region (V), low fungal abundances were found. *Trichoderma* strains were isolated on two different selective media TSM and TSCM (Smith *et al.,* 1990; Elad and Chet, 1983) but on both media strains belonging to other fungal genera were grown. From all vegetation zones, *Trichoderma* colonies could be isolated. In general, on TSM, lower averages were found ($\log_{10}$ 3.97 CFU per g sfw) than on TSCM ($\log_{10}$ 5.69 CFU per g sfw). Again, no statistically significant differences between the sampling points were found.

As a standard for absolute quantification, a cloned ITS1/2 fragment of an environmental *T. reesei* sequence was used in 10-fold dilutions from 10^5 to 10^2. Calculation of the standard curve showed an r^2 of 0.94 for the real-time PCR (data not shown). Sample curves crossed the threshold at a C_t value of 17–36 resulting in a mean copy number of $\log_{10}$ 4.7–8.4 CFU per g sfw. Again, in all samples, *Trichoderma* DNA was found; the abundances were similar to each other and no statistically significant effect could be measured.

Owing to poor identification of *Trichoderma* found with direct clone sequencing, *Trichoderma* strains isolated from the different sampling points were characterized according to an established barcoding procedure (Druzhinina *et al.*, 2005). A combination of ITS1/2 and *tef*1 sequencing identified 22 representative isolates as *T. harzianum* (13), *T. spirale* (1), *T.* cf. *tomentosum* (1), *T. gamsii* (3), *T. viridescens* (1), *T. viride* (1) and *T. 'viride* Ve' (2) (Table 4). The latter represents an as-yet-undescribed phylogenetic species from the *H. rufa* clade that is so far known only from countries of the Pacific rim (Jaklitsch *et al.*, 2006). The genotypic diversity of these *Trichoderma* strains was also investigated by BOX fingerprints, which revealed a higher intraspecific diversity and zone-specific genotypes. Three different molecular patterns were found for *T. harzianum* strains isolated from three different locations (Supplementary Figure S2). In addition, two different genotypes of *T. gamsii* were found, one was originated from Laurisilva (L) and the other one from the succulent zone (S).

All strains were tested for their *in vitro* antagonism towards various plant pathogens. The selected *Trichoderma* isolates were screened for their antagonistic ability to suppress the growth of *B. cinerea, G. bidwellii, R. solani* AG2-2IIIB and AG4, *S. rolfsii*, and *V. dahliae*. Interestingly, all of them showed an extremely high antagonistic activity.

Table 2 Identification and taxonomic classification of fungal DNA bands separated by SSCP from different sampling locations in Tenerife Island

OTU	*Location*[a]	*ITS band*[b]	*Closest NCBI database match*	*Accession no.*	*SI*[c]	*Taxonomic grouping*
OTU4	D1	ITS1	*Rhizoctonia* sp.	AF200520.1	84	Basidiomycota
OTU8	D1	ITS2	*Tulostoma kotlabae* voucher MJ6623	DQ112629.1	92	Basidiomycota
OTU21	D1	ITS3	*Thanatephorus* sp. CBS 320.84	DQ278949.1	84	Basidiomycota
OTU13	D2	ITS5	Basidiomycete from a bamboo (*Arundinaria gigantea*)	U65619.1	84, 85	Basidiomycota
OTU10	D2	ITS7	Uncultured soil fungus clone 53-1	DQ421097.1	89	Fungus
OTU13	D2	ITS8	*Polyporus ciliatus*	AB070882.1	87	Basidiomycota
OTU5	L1	ITS17	Uncultured fungus clone L5_OTU39	EF434028.1	90	Fungus
OTU10	L1	ITS18	*Ganoderma australe*	AY884180.1	94	Basidiomycota
OTU19	L1	ITS19	*Entoloma bloxamii*	EF530938.1	83	Basidiomycota
OTU21	L1	ITS20	*Rhizoctonia* sp. 3-4Ll20-5p	AM040889.1	87	Basidiomycota
OTU12	L2	ITS21	Hygrocybe conica	AY854074.1	93	Basidiomycota
OTU14	L2	ITS23	*Hygrocybe conica* isolate AFTOL-ID 729	AY854074.1	96	Basidiomycota
OTU15	L2	ITS24	*Marasmius* sp. NC-8330/1	AY456352.1	91	Basidiomycota
OTU35	L2	ITS137	*Cryptococcus podzolicus*	AF444321.1	98	Basidiomycota
OTU13	P1	ITS27	*Mallocybe dulcamara* isolate AFTOL-ID 482	DQ221106.1	81	Basidiomycota
OTU1	P1	ITS28	*Hebeloma cistophilum*	DQ007992.1	98	Basidiomycota
OTU6	P1	ITS29	*Hebeloma cistophilum* voucher GLM 62250	DQ007993.1	98	Basidiomycota
OTU10	P1	ITS30	*Inocybe nitidiuscula*	AM882911.1	97	Basidiomycota
OTU12	P2	ITS31	Uncultured ectomycorrhiza (Inocybe) isolate UBCOCS196F	EF218776.1	94	Basidiomycota
OTU13	P2	ITS32	*Inocybe* cf. *glabripes*	AJ889952.1	90	Basidiomycota
OTU12	R1	ITS34	*Polyporus ciliatus*	AB070882.1	87	Basidiomycota
OTU13	R1	ITS35	*Cryptococcus phenolicus*	AF444351.1	98	Basidiomycota
OTU12	R1	ITS36	*Cryptococcus bhutanensis*	EU266557.1	99	Basidiomycota
OTU15	R1	ITS37	*Cryptococcus aerius*	AF145324.1	97	Basidiomycota
OTU13	R2	ITS38	*Cryptococcus phenolicus* strain CBS 8682	AF444351.1	96	Basidiomycota
OTU5	R2	ITS39	*Trechispora alnicola* isolate AFTOL-ID 665	DQ411529.1	77	Basidiomycota
OTU12	R2	ITS40	*Cryptococcus bhutanensis* strain CBS6294	AF145317.1	97	Basidiomycota
OTU16	R2	ITS41	*Cryptococcus aerius*	AF145324.1	99	Basidiomycota
OTU16	S1	ITS9	*Cryptococcus aerius* strain CBS155	AF145324.1	97	Basidiomycota
OTU21	S1	ITS10	Uncultured soil basidiomycete clone B4	DQ672292.1	91	Basidiomycota
OTU14	S1	ITS11	*Nolanea sericea*	AF357021.2	83	Basidiomycota
OTU25	S1	ITS12	Uncultured soil basidiomycete.	AY969789.1	88	Basidiomycota
OTU22	S1	ITS120	Uncultured soil basidiomycete	DQ672292.1	90	Basidiomycota
OTU8	S1	ITS122	Uncultured soil basidiomycete	DQ672292.1	89	Basidiomycota
OTU5	S1	ITS125	Uncultured basidiomycete	EU003034.1	73	Basidiomycota
OTU23	S1	ITS126	Uncultured endophytic fungus	EF505775.1	86	Fungus
OTU19	S2	ITS13	Uncultured Auriculariales	EF619638.1	93	Basidiomycota
OTU13	S2	ITS14	*Rhizoctonia* sp. 3-4Ll20-5p	AM040889.1	84	Basidiomycota
OTU15	S2	ITS15	*Psilocybe* sp.	DQ002870.1	77	Basidiomycota
OTU22	S2	ITS16	Uncultured basidiomycete isolate dfmo0724_065	AY969789.1	80	Basidiomycota
OTU7	S2	ITS128	Uncultured fungus	DQ093748.1	96	Fungus
OTU22	S2	ITS130	*Sphaerobolus* sp.	DQ979014.1	77	Basidiomycota
OTU17	V1	ITS46	*Cryptococcus albidus* var. *ovalis*	EU266553.1	98	Basidiomycota
OTU22	V1	ITS47	*Cryptococcus vishniacii*	DQ317364.1	98	Basidiomycota
OTU8	V1	ITS48	*Thelebolaceae* sp.	DQ317351.1	100	Ascomycota
OTU15	V1	ITS49	*Cryptococcus friedmannii*	DQ317360.1	92	Basidiomycota
OTU17	V1	ITS50	*Cryptococcus albidus*	EU266553.1	99	Basidiomycota
OTU26	V1	ITS106	*Erythrobasidium clade* sp.	EF060907.1	97	Basidiomycota
OTU29	V1	ITS107	*Naohidea sebacea*	DQ911616.1	84	Basidiomycota
OTU8	V2	ITS51	*Thelebolaceae* sp.	DQ317351.1	99	Ascomycota
OTU12	V2	ITS52	*Cryptococcus antarcticus*	AB032670.1	96	Basidiomycota

Abbreviation: SSCP, single-strand conformation polymorphism.
[a]Letters represent the locations D1, D2, L1, L2, P1, P2, R1, R2, S1, S2, V1 and V2 (see Table 1).
[b]Number of band (see Figure 2).
[c]SI, similarity index: for isolates identified by ITS1 and ITS2 fragment (fungi) sequencing ranging from 0% to 100%.

Discussion

The Canary Islands have proven to be an interesting object for studying biodiversity and diversification of species. Our model Tenerife Island is the most diverse of the Canary Islands, containing a high number of bioclimatic belts and zonal habitats in a small geographical area. In these zones, highly specific plant communities exists, and a high degree of endemic plants occurred (Francisco-Ortega *et al*., 2000). Furthermore, special soil types have been developed for each vegetation zone from the volcanic material due to the effects of vegetation, local weather conditions and topography

Table 3 Identification and taxonomic classification of *Trichoderma* DNA bands separated by SSCP from different sampling locations in Tenerife Island

OTU	*Location*[a]	*Trichoderma band*[b]	*Closest NCBI Database match and accession number*	*Accession no.*	*SI*[c]
OTUE	D2	T1	*Hypocrea lixii* isolate UOKT044[d]	EF442082.1	98
OTUE	L1	T5	*Hypocrea lixii* isolate UOKT044	EF442082.1	99
OTUL	L1	T6	*Trichoderma viride* isolate GJS 99-83[e]	AF456921.1	96
OTUL	L1	T64	*Trichoderma viride* GJS 99-83[e]	AF456.921.1	98
OTUE	L2	T7	*Trichoderma spirale* isolate aurim1210	DQ093712.1	98
OTUL	L2	T8	*Trichoderma viride* isolate GJS 99-83	AF456921.1	96
OTUD	L2	T9	*Hypocrea lixii* isolate UOKT044	EF442082.1	99
OTUL	L2	T10	*Trichoderma viride* isolate GJS 99-83[e]	AF456921.1	96
OTUA	P1	T11	*Hypocrea lixii* isolate UOKT044	EF442082.1	98
OTUC	P1	T12	*Hypocrea lixii* isolate UOKT04	EF442082.1	100
OTUC	P1	T13	*Hypocrea lixii* strain T-18[f]	DQ023715.1	90
OTUH	P1	T55	*Hypocrea lixii* strain VI03698	AM498496.1	99
OTUH	P1	T24	*Trichoderma hamatum* isolate TUB F-617	AF486013.1	75
OTUE	P1	T36	*Hypocrea lixii* isolate UOKT044	EF442082.1	99
OTUA	P2	T14	*Hypocrea lixii* isolate UOKT044	EF442082.1	98
OTUC	P2	T15	*Hypocrea lixii* isolate UOKT044	EF442082.1	99
OTUE	P2	T25	*Hypocrea lixii* isolate UOKT044	EF442082.1	98
OTUF	P2	T26	*Hypocrea lixii* isolate UOKT044	EF442082.1	99
OTUF	R1	T16	*Hypocrea lixii* isolate UOKT044	EF442082.1	99
OTUE	R1	T27	*Hypocrea lixii* isolate UOKT044	EF442082.1	97
OTUE	R1	T28	*Hypocrea lixii* isolate UOKT044	EF442082.1	98
OTUF	R1	T73	*Trichoderma tomentosum* strain UNISS 13b-12	EF488143.1	96
OTUE	R2	T29	*Hypocrea lixii* isolate UOKT044	EF442082.1	99
OTUE	R2	T30	*Hypocrea lixii* isolate UOKT044	EF442082.1	99
OTUL	R2	T31	*Trichoderma hamatum*	DQ682602.1	97
OTUD	S1	T2	*Trichoderma velutinum*	DQ083010.1	99
OTUK	S1	T67	*Trichoderma atroviride* strain VI03932	AM498487.1	98
OTUD	S2	T3	*Hypocrea lixii* isolate UOKT044	EF442082.1	98
OTUD	S2	T4	*Hypocrea lixii* isolate UOKT044	EF442082.1	99
OTUC	S2	T69	*Hypocrea cremea* strain GJS 91-125	AY737760.1	100
OTUE	S2	T70	*Hypocrea lixii* strain VI03698	AM498496.1	100
OTUG	V1	T18	*Hypocrea lixii* isolate UOKT044	EF442082.1	99
OTUG	V1	T19	*Hypocrea lixii* isolate UOKT044	EF442082.1	93
OTUE	V1	T57	*Hypocrea lixii* strain VI03698	AM498496.1	100
OTUF	V1	T59	*Hypocrea lixii* strain VI03698	AM498496.1	100
OTUE	V1	T34	*Hypocrea lixii* isolate UOKT044	EF442082.1	97
OTUF	V2	T20	*Hypocrea lixii* isolate UOKT044	EF442082.1	97
OTUG	V2	T21	*Hypocrea lixii* isolate UOKT044	EF442082.1	98
OTUF	V2	T22	*Hypocrea lixii* isolate UOKT044	EF442082.1	99
OTUG	V2	T23	*Hypocrea lixii* isolate UOKT044	EF442082.1	98
OTUE	V2	T62	*Trichoderma tomentosum* strain UNISS 13b-12	EF488143.1	100
OTUE	V2	T35	*Hypocrea lixii* isolate UOKT044	EF442082.1	98

Abbreviation: SSCP, single-strand conformation polymorphism.
[a]Letters represent the locations D1, D2, L1, L2, P1, P2, R1, R2, S1, S2, V1 and V2 (see Table 1).
[b]Number of band (see Supplementary S6).
[c]SI, similarity index: for isolates identified by uTf and ITS4 fragment (*Trichoderma*) sequencing ranging from 0% to 100%.
[d]*T. stromaticum*
[e]*Trichoderma* sp.
[f]*Hypocrea* sp. according to TrichoBLAST (www.isth.info)

(Fernández Caldas *et al*., 1987; Hernández-Moreno *et al*., 2007). For example, Canarian endemic pine forests grow on two kinds of fertile soil: ancient red lands and grey clay lands with low organic material content formed in places with well-defined seasons. Heathlands are located in humid zones in young soils composed of hydrated aluminic silicates with a high level of organic material, whereas Laurisilva occurs on recent materials in places with well-defined seasons and medium-to-low organic substance levels, which lie above older soil types. Our correspondence analysis data show that each vegetation zone is characterized by specific fungal diversity data: they were very similar between both sampling sites on the island, although long distances between the sampling points mostly exist. The similarity of the results obtained by CA and direct correspondence analysis indicate a high stability of fungal communities. For Laurisilva, the famous old evergreen Laurel wood (L), which is known for its extraordinary and deviant flora, the fungal data show the highest distance within the data set. Altogether, fungal communities followed the same environmental gradients from arid to semiarid and from high to low temperature like the vascular plant vegetation.

Trichoderma is a key fungal genus in agricultural soils responsible for soil and plant health (Harman

Table 4 Identification of *Trichoderma* strains isolated from sampling locations in Tenerife Island and cultivated on selective media with *in vitro* antagonistic potential towards various plant pathogens on potato dextrose agar

Isolate	*Location*	*Closest NCBI database match and accession number*	*Genotype*	*Antagonistic potential against*					
				Botrytis cinerea	Guignardia bidwellii	Rhizoctonia solani *AG2-2IIIB*	Rhizoctonia solani *AG4*	Sclerotium rolfsii	Verticillium dahliae *V25*[a]
TR1[b]	D2	*Trichoderma harzianum*	1	+++	+++	++	++	+++	+++
TR2[b]	D2	*Trichoderma harzianum*	1	+++	+++	+++	+++	+++	+++
TR3[c]	D2	*Trichoderma harzianum*	1	+++	+++	+++	+++	+++	+++
TR4[b]	D2	*Trichoderma harzianum*	1	+++	+++	+++	+++	+++	+++
TR5[d]	D2	*Trichoderma harzianum*	1	+++	+++	+++	+++	+++	+++
TR6[d]	D2	*Trichoderma harzianum*	1	+++	+++	+++	+++	+++	+++
TR7[d]	D2	*Trichoderma harzianum*	1	+++	+++	+++	+++	+++	+++
TR8[b]	R2	*Trichoderma chionea*	2	+++	+++	+++	+++	+++	+++
TR9[b]	P1	*Trichoderma cf. tomentosum*	3	+++	+++	+++	+++	+++	+++
TR10[b]	S2	*Trichoderma gamsii*	4	+++	+++	+++	+++	+++	+++
TR11[b]	S2	*Trichoderma gamsii*	5	+++	+++	+++	+++	+++	+++
TR12[b]	P2	*Trichoderma harzianum*	6	+++	+++	+++	+++	+++	+++
TR13[b]	L1	*Trichoderma gamsii*	7	+++	+++	+++	+	+++	++
TR14[b]	L1	*Trichoderma chionea*	8	—	—	—	—	—	—
TR15[d]	L1	*Trichoderma harzianum*	9	+++	+++	+++	+++	+++	+++
TR16[b]	L2	*Trichoderma harzianum*	9	+++	+++	+++	+++	+++	+++
TR17[b]	L2	*Trichoderma harzianum*	9	+++	+++	+++	+++	+++	+++
TR18[b]	L2	*Trichoderma harzianum*	9	+++	+++	+++	+++	+++	+++
TR19[b]	L2	*Trichoderma spirale*	10	+++	+++	+++	+++	+++	+++
TR20[b]	L2	*Trichoderma harzianum*	11	+++	+++	+++	+++	+++	+++
TR21[b]	L2	*Hypocrea rufa*	12	+++	+++	+++	+++	+++	+++

[a]Dual culture on Waksman agar.
[b]Isolated from potato dextrose medium.
[c]Isolated from *Trichoderma* selective medium.
[d]Isolated from modified *Trichoderma* selective medium.
+ 0–5 mm, ++ 5–10 mm, +++ >10 mm radius of zone of hyperparasitism in dual culture assay; — no suppression.

et al., 2004; Berg *et al.*, 2005). On Tenerife Island, in contrast, only low diversity in *Trichoderma* populations was found. The diversity was much lower than those reported for the rhizosphere of agricultural soils and crops; for example, as reported by Berg *et al.* (2005) or in South-East Asia (Kubicek *et al.*, 2003) and South America (Druzhinina *et al.*, 2005). All indigenous *Hypocrea/Trichoderma* species are cosmopolitan with an extraordinarily high antagonistic potential against fungal pathogens, and many of them are already known from many locations in Eurasia and Africa (Kubicek *et al.*, 2003). In comparison with the whole fungal community, *Trichoderma*-specific communities showed a low diversity and no correlation to plant communities or abiotic factors. Therefore, this study presented different ecological and evolutionary strategies of fungi.

Our study showed that the cultivation-independent approach was able to analyse *Trichoderma* diversity on ITS level but was unable to unambiguously identify *Trichoderma* at the species level. Hagn *et al.* (2007) developed primers that are able to amplify an approximately 540-bp fragment comprising ITS1, 5.8S rDNA and ITS2 from all taxonomic clades of the genus *Trichoderma*. They are useful for quantitative PCR and fingerprint analysis but do not fit to identification systems like *Trichoderma*Key 2.0 (Druzhinina *et al.*, 2005). Identification of strains, isolated by cultivation on plates, resulted in a pattern partially different from the cultivation-independent approach; although *T. harzianum* was identified as the dominant species, the two ITS1 and -2 alleles were different from those obtained from direct sequencing. One of the two alleles of the isolated strains resembled the one only found in Europe (B9b), whereas the other one (B7a) is cosmopolitan. A similar predominance of the B9b allele was recently found on the island of Sardinia (Migheli *et al.*, 2008). Also *T. spirale*, *T.* cf. *tomentosum* and *T. gamsii* had been found to be abundant in Sardinia. In contrast to the high specificity found for the whole fungal community, Tenerife is mainly colonized by ubiquitous and widely distributed *Trichoderma* species and not by endemics. The high antagonistic spectrum and competitiveness of the *Trichoderma* isolates of Tenerife underlined the hypothesis that the *Hypocrea/Trichoderma* community is suppressed by invasive strains from other continents. They can be transmitted through wind as a long distance vehicle, for example strong Passat wind known for this region, because fungal spores have been detected in the stratosphere (Muñoz *et al.*, 2004).

The fungal communities were characterized by a high diversity and dominance of Basidiomycota, and a high degree of unidentified species. Further-

more, many of the sequenced clones had a high similarity with clones from uncultivated, unknown fungi. However, for those that we could attribute to a genus, many of them are known to form ectomycorrhizae, an old symbiosis between fungi and plants, which help plants to obtain mineral nutrients from the soil. A similar combination of species was found by Obase *et al*. (2007), studying the development of ectomycorrhizae at a recently erupted volcano (Mount Usu, Japan). Another notable finding of this study was the abundance of species of the basidiomycetous yeast *Cryptococcus*. Isolates of *Cryptococcus* spp. were also detected as the major fungal population in the soil of natural areas in some other studies too (Wuczkowski and Prillinger, 2004; Vishniac, 2006; Connell *et al*., 2008). The predominance of *Cryptococcus* species in soils has been attributed to the unique polysaccharide capsules surrounding those (McFadden *et al*., 2006) that aid in the assimilation of nutrients from soil and thus competing with bacteria and other fungi in arid soils (Vishniac, 2006).

The functioning and stability of terrestrial ecosystems are determined by biodiversity and species composition (McCann, 2000). However, the ecological mechanisms by which biodiversity and species composition are regulated and maintained are not well understood. Interestingly, there are reports showing the effect of fungal communities on plant communities and vice versa (van der Heijden *et al*., 1998). Recent empirical analyses of diversity patterns of microorganisms suggest that there are biodiversity scaling rules common to all forms of life (Green and Bohannan, 2006). In our study, we could show a high correlation between plant and fungal communities and environmental factors. The fungal communities on Tenerife Island are models that follow the same rules as the plant communities. The high proportion of isolates, which could not be identified, gives an additional hint of new and endemic species. The close link between plant species and microbial community structure in the rhizosphere resulted in the development of unique fungal diversity pattern over time and showed a parallel microbial evolution.

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