RESEARCH ARTICLE

Succession of N cycling processes in biological soil crusts on a Central European inland dune

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Abstract

Biological soil crusts (BSCs) are microbial assemblages that occur worldwide and facilitate ecosystem development by nitrogen (N) and carbon accumulation. N turnover within BSC ecosystems has been intensively studied in the past; however, shifts in the N cycle during BSC development have not been previously investigated. Our aim was to characterise N cycle development first by the abundance of the corresponding functional genes (in brackets) and second by potential enzyme activities; we focussed on the four processes: N fixation (*nifH*), mineralisation as proteolysis and chitinolysis (*chiA*), nitrification (*amoA*) and denitrification (*nosZ*). We sampled from four phases of BSC development and from a reference located in the rooting zone of *Corynephorus canescens*, on an inland dune in Germany. BSC development was associated with increasing amounts of chlorophyll, organic carbon and N. Potential activities increased and were highest in developed BSCs. Similarly, the abundance of functional genes increased. We propose and discuss three stages of N process succession. First, the heterotrophic stage (mobile sand without BSCs) is dominated by mineralisation activity. Second, during the transition stage (initial BSCs), N accumulates, and potential nitrification and denitrification activity increases. Third, the developed stage (established BSCs and reference) is characterised by the dominance of nitrification.

Introduction

Biological soil crusts (BSCs) are microbial communities generally composed of bacteria, archaea, cyanobacteria, fungi, algae, mosses and lichens (Belnap & Lange, 2001) that colonise soil top layers. BSCs are highly stress tolerant under extreme environmental conditions and can therefore be found globally in a variety of ecosystems, including deserts and polar regions (Redfield *et al.*, 2002; Nagy *et al.*, 2005; Budel & Veste, 2008; Schmidt *et al.*, 2008; Ben-David *et al.*, 2011). In ecosystems, such as volcanic areas, glacier forefields and flood plains, BSC forms the basis for further ecosystem development (Eldridge & Tozer, 1996; Cutler *et al.*, 2008; Yoshitake *et al.*, 2010).

Depending on the type of ecosystem, the expression development has to be interpreted with care. In systems affected by volcanic eruption, the soil surface may recover simultaneously at different locations (Vitousek *et al.*, 1983; Crews *et al.*, 2001). In the forefield of a receding glacier, a chronosequence can be observed, and the phase of BSC development depends on the time span since deglaciation (Chapin *et al.*, 1994). In a sand dune, shear forces by wind and sand exert stress at the surface and so affect BSC development. The shear stress is very high on the top of the sand dune and decreases along the lee slope (Kroy *et al.*, 2002). Therefore, BSC development is not necessarily a function of time, but rather a function of distance from the top of the dune.

Nitrogen (N) is a limiting factor in initial ecosystems, because it is an essential macronutrient and initially absent from the soil. Therefore, N supply to the initial ecosystem is crucial for further development. One important input pathway is the biological N fixation that

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was shown to be the dominant process providing N to BSC (Rychert & Skujins, 1974; West & Skujins, 1977; Zaady, 2005). Within the BSC, three microbially mediated processes dominate the N cycle: mineralisation, nitrification and denitrification. All N turnover processes depend on the availability of N, which is closely linked to soil water content and soil hydrology. Moreover, atmospheric N deposition by precipitation and N leaching from the soil are important input and output pathways that are closely linked to soil hydrology. Therefore, soil hydrology needs to be taken into account when assessing the N cycle.

N turnover processes in BSCs have been assayed by a variety of methods (for an overview see West & Skujins, 1978; Belnap & Lange, 2001), and most methods are well established with standardised protocols (Jeffries *et al.*, 1992; Luo *et al.*, 1996; Hendel & Marxsen, 2005; Hoffmann *et al.*, 2007). Within the last few years, however, molecular tools have become increasingly reliable. Using quantitative real-time PCR (qPCR), the abundance of functional genes in soil can be measured (Henry *et al.*, 2004; Yeager *et al.*, 2004) to assess the genetic potential of a microbial community catalysing the process of interest. qPCR assays for many genes involved in the N cycle are established (Brankatschk *et al.*, 2011), namely nitrogenase (*nifH*), chitinase (*chiA*), protease (*aprA*), ammonia monooxygenase (*amoA*) of ammonia oxidising archaea (AOA), *amoA* of ammonia oxidising bacteria (AOB) and nitrous oxide reductase (*nosZ*).

To date, numerous studies have focused on individual N processes (Barger *et al.*, 2005; Johnson *et al.*, 2005), N redistribution (Zaady, 1998; Johnson *et al.*, 2007) and N balance of BSC systems (West & Skujins, 1977; Zaady, 2005). Other studies have compared the N cycle in different types of BSCs (Evans & Belnap, 1999; Zaady *et al.*, 2010; Strauss *et al.*, 2011). Efforts to study the development of the N cycle have concentrated on systems such as volcanic areas (Vitousek *et al.*, 1983; Crews *et al.*, 2001) and glacier forefields (Chapin *et al.*, 1994; Brankatschk *et al.*, 2011), but BSC development has been neglected.

The aim of this study was to investigate the N cycle during BSC development, and the Lieberose inland dune (Germany) was chosen as study site (Fischer *et al.*, 2010). Our first hypothesis was that the Lieberose dune displays the full spectrum of BSC development from mobile sand to established BSC. Second, we hypothesised that the development of the N cycle in the studied BSC system proceeds in stages. To test the hypotheses, we analysed the major N cycling processes: N fixation, mineralisation (proteolysis and chitinolysis), nitrification and denitrification, employing standard enzyme assays and novel qPCR techniques.

Materials and methods

Study site

The study site is located on a Central European inland dune on Pleistocene sand (Fischer *et al.*, 2010) near Lieberose, Brandenburg, Germany (51°55′49″N, 14°22′22″E). In this area, an initial mobile sand dune occurs as a result of human disturbance of the natural vegetation by former military activities. The climate of the region is characterised by an annual rainfall of 570 mm and an annual average temperature of 8.9 °C (Fischer *et al.*, 2010). A private meteorological station (WS 2500; ELV Elektronik AG, Germany) is maintained in the settlement of Hollbrunn, located 2 km north-west of the study site (http://www.hollbrunn-wetter.de/). Daily precipitation and average daily temperature for 2010 are shown in Fig. 1a. The annual atmospheric N deposition is 12–15 kg ha^{-1} a^{-1} (Gauger *et al.*, 2008, http://gis.uba.de/website/depo_gk3/index.htm).

Modelling soil hydrology

Using the Hydrus-1D modelling environment (Radcliffe & Šimůnek, 2010), we calculated evaporation from the soil surface and the water flux in the soil. Evaporation from the dune was estimated using the Penman–Monteith equation, as implemented in Hydrus-1D (Radcliffe & Šimůnek, 2010). The calculation was based on air temperature, wind speed, relative humidity and sun hours (data not shown). Water flux on the soil surface and at 25 cm depth was calculated by solving the Richard's equation in the one-dimensional finite element model implemented in Hydrus-1D (Radcliffe & Šimůnek, 2010).

BSCs at the study site

At the Lieberose dune, different phases of BSC development can be observed (Fischer *et al.*, 2010, 2012). On the top of the dune, wind keeps the sand mobile, and no BSC develops because of frequent disturbances owing to shear stress. However, on the lee slope of the dune, disturbance by wind gradually decreases. This disturbance gradient down the lee slope causes the persistence of different phases of BSC development, which lie 5–10 m to one another. At the foot of the dune, the lowest level of disturbance is observed. We sampled four phases of BSC development, that is, mobile sand, phase 1, phase 2 and phase 3, which were previously characterised (Fischer *et al.*, 2010, 2012). The mobile sand is the bare substrate with no BSC development. BSC of phase 1 consists of sand grains that are stabilised at their contact zones by filamentous cyanobacteria and green algae. BSC of phase

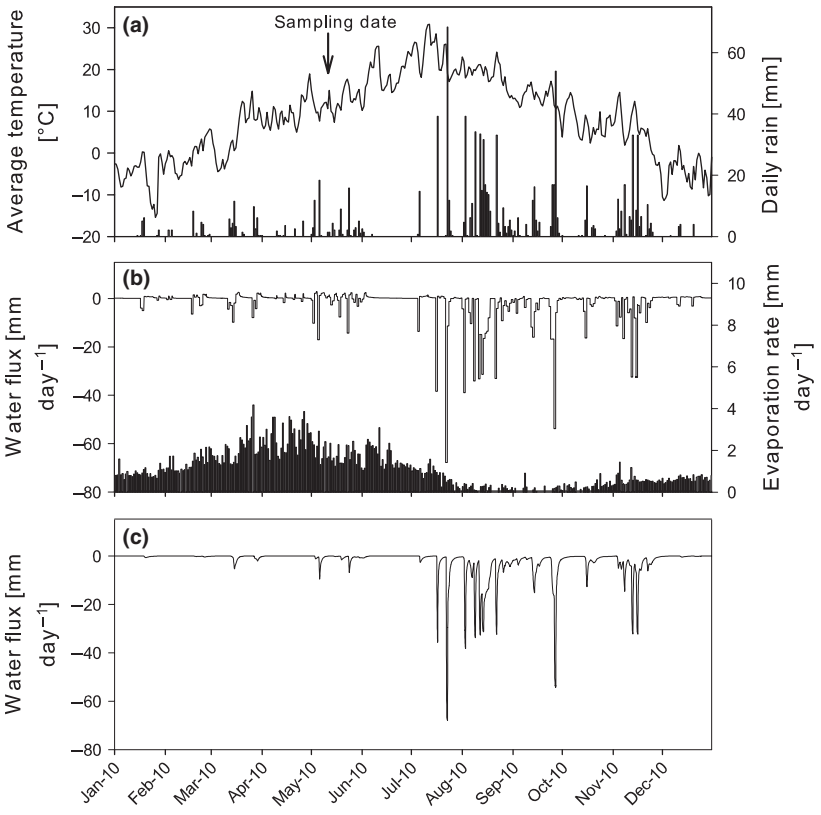

Fig. 1. Temperature, precipitation, water fluxes and evaporation for the year 2010. (a) Average air temperature (solid line) and daily rain (bars) measured at the private meteostation in Hollbrunn (http://www.hollbrunn-wetter.de/). (b) Modelled water flux (solid line) and evaporation (bars) on soil surface. (c) Modelled water flux in 25 cm depth.

2 is characterised by cyanobacteria and green algae, which partially fill matrix pores and enmesh with sand grains. BSC of phase 3 shows intense growth of filamentous and coccoid green algae, and a few cyanobacteria, fungal hyphae and mosses (Fischer *et al.*, 2010). The flora of the BSCs at the Lieberose dune was described earlier (Fischer *et al.*, 2012). BSC depth increased from about 1 mm in phase 1 to up to 4 mm in phase 3 (see Supporting information, Figs S1-S4). Together with the increasing surface stability and the development of the BSCs, the grass *Corynephorus canescens* establishes, which is characteristic for early successional stages of dry grassland ecosystems on Central European sand dunes (Fig. S5). The highest grass cover (17%) can be found at the dune base together with crust 3.

Sampling procedure

All four phases of BSC development (mobile sand, phase 1, phase 2 and phase 3) were sampled on May 12, 2010. The crusts (C) of each phase were sampled by pressing Petri dishes into the topsoil. The subsoils (5–15 mm, S) and the deeper subsoils (15–35 mm, D) of each phase were sampled using shovels. Five independent samples of each phase of BSC development were sampled 1–2 m apart. Each sample was a composite sample of five Petri dishes (8.7 cm diameter) or shovels taken within 0.5 m^2. In addition, a reference soil (R) from the rooting zone (50–100 mm depth) of *C. canescens* was sampled in five replicates at the base of the dune, near phase 3. The rhizosphere was chosen as reference because it is influenced by higher plants and represents a vegetated soil, in contrast to BSC, which is void of higher plants (Langhans *et al.*, 2009).

In addition to the samples mentioned previously, cored samples (1 cm depth) of the crusts were taken in triplicates with a corer (Fischer *et al.*, 2010) close to the other samples. These cored samples were used to measure chlorophyll content and repellency index.

Samples for molecular analysis were aliquoted, kept on ice in the field and stored at −80 °C upon arrival in the laboratory. All other samples were air-dried in the laboratory and kept dry and in the dark until analysis.

Soil chemical and physical analysis

Total organic carbon (TOC) was determined as total carbon, as the samples contained no carbonates. Total nitrogen (TN) and total carbon were measured on an elemental analyser (vario EL III; Elementar Analysensysteme GmbH, Hanau, Germany). Soil nitrate, ammonium and pH were analysed in water extracts 1 : 2.5 (w/w). Nitrate was measured on a Dionex ICS 90 and ammonium

on a Dionex DX 100 (both Dionex, Sunnyvale, CA). Chlorophyll extracts were prepared with 80% acetone, as previously described (Fischer *et al.*, 2010). Chlorophyll *a* and *b* content was calculated from the absorption spectra of the acetone extracts (Ziegler & Egle, 1965). A laser Diffraction Particle Size Analyser LS 13 320 (Beckman Coulter, Miami, FL) fitted with an Aqueous Liquid Module and an Auto Prep Station was used to determine the particle size distribution. Sand was classified as medium sand with 10–30% coarse sand (630–2000 μm), 55–62% medium sand (200–630 μm), 15–20% fine sand (63–200 μm) and < 4% of particles below 63 μm in diameter.

Potential enzyme activities

We choose to employ potential enzyme activity assays, as they are a robust indication for the microbial functional capacity, reflecting activity rates under ideal conditions, for example, as was shown for denitrification assay in BSCs (Johnson *et al.*, 2007). Potential N fixation activity was measured by acetylene reduction assays (Jeffries *et al.*, 1992) (Table 1). As a proxy for mineralisation activity (Schimel & Bennett, 2004), potential chitinase and protease activity were measured in fluorescence assays (Hendel & Marxsen, 2005). Potential nitrification activity was measured as previously described (Hoffmann *et al.*, 2007). Potential denitrification assays were performed as reported by Luo *et al.* (1996). All enzyme assays were performed in triplicates. The limit of detection (LOD) was calculated as the mean of six blank reactions plus three times standard deviation of the blanks. The limit of quantification (LOQ) was calculated as the mean of six blank reactions plus 10 times standard deviation of the blanks.

DNA extraction and real-time PCR

DNA was extracted from 0.5 g of thawed BSC or soil material using the Fast DNA Spin Kit for Soil (MP Biomedicals, Illkirch, France) and eluted in 60 μL of TE buffer. DNA yields were quantified using the SYBR Green I assay as described by Matsui *et al.* (2004).

SYBR Green based real-time PCR assays were run on a 7300 real-time PCR system (Applied Biosystems, Foster City, CA). Reaction volume was 20 μL and contained onefold concentrated Kapa SYBR Fast PCR master mix (Kapa Biosystems, LabGene, Chatel-St-Denis, Switzerland), 200 nM of each of the respective forward and reverse primers and 1 μL DNA template (1 : 10 diluted DNA extract, optimal dilution without inhibition). PCR primers, thermal cycles, efficiencies, LOQs and calibration standards used are summarised in Table 2. Each PCR run included triplicate sample templates, calibration standards and no template control (PCR grade water). Copy numbers were calculated by the one-point calibration (OPC) method (Brankatschk *et al.*, 2012) using LINREGPCR program version 11.4 to calculate mean efficiency of each sample (Ruijter *et al.*, 2009). Any outliers detected in LINREGPCR analysis (no amplification) were excluded. The specificity of the amplification was verified by agarose gel electrophoresis. If a desired amplicon was not detected, samples were reported as not detected. Assuming one PCR template will produce the desired band, the theoretical LOD of our set-up was 1.2×10^3 copies g^{-1}. The LOQ was calculated as mean of five or more reactions without desired amplicon (amplicon-negative samples or blanks) plus 10 times the standard deviation.

Results

Soil physical and chemical characterisation

Water flux at the surface was variable (Fig. 1b). At 25 cm depth, it was less variable than at the soil surface (Fig. 1b and c), and not every precipitation event caused a negative water flux at this depth.

Soil chemistry was distinctly different for the BSC phases. TOC, TN and ammonium content in the crusts generally increased from mobile sand to phase 3 (Fig. 2).

Table 1. Conditions and references of the performed potential enzyme assays

Process (Assay)	Amount of sample (g)	Incubation time and temperature	LOD; LOQ	Reference
N fixation (acetylene reduction assay)	2	20 h at 23 °C*	1; 3 pmol C_2H_4 g^{-1} h^{-1}	Jeffries *et al.* (1992)
Mineralisation (chitinase assay)	0.5	6 h at 23 °C†	6; 24 pmol MUF g^{-1} h^{-1}	Hendel & Marxsen (2005)
Mineralisation (protease assay)	0.5	6 h at 23 °C†	8; 28 pmol MUF g^{-1} h^{-1}	Hendel & Marxsen (2005)
Nitrification (potential nitrification assay)	5	5 h at 25 °C	0.6; 0.9 nmol NO_2^--N g^{-1} h^{-1}	Hoffmann *et al.* (2007)
Denitrification (potential denitrification assay)	5	16 h at 20 °C‡	10; 20 pmol N_2O-N g^{-1} h^{-1}	Luo *et al.* (1996)

*Light intensity (30 μmol photons m^{-2} s^{-1}), constant rate for 20–72 h of incubation.

†50 mM acetate buffer, 10 μM fluorescence substrate.

‡0.1 mM glucose, 0.1 mM potassium nitrate, and 0.1 g L^{-1} chloramphenicol, constant rate from 14 to 48 h.

Table 2. Temperature cycles, mean efficiencies, sources of calibration standards and primers of qPCRs

	Temperature cycles*						
Target gene	Denaturation time at 95 °C (s)	Annealing time and temperature	Elongation time at 72 °C (s)	Efficiency† (%)	LOQ (copies g^{-1})	Source of calibration standard	Primer name and reference
nifH	15	25 s at 53 °C‡	45	75–79	7×10^5	*Paenibacillus durus* DSM5976	nifH-F, nifH-R, Rösch *et al.* (2002)
chiA	10	90 s at 65 °C	30	79–82	2×10^3	*Streptomyces coelicolor* DSM41189	chif2, chir, Xiao *et al.* (2005)
aprA	10	20 s at 54 °C	30	70–72	7×10^5	*Pseudomonas fluorescens* DSM 50090	FR apr I, RP apr II, Bach *et al.* (2001)
nosZ	15	30 s at 58 °C§	30	77–79	2×10^3	*Pseudomonas fluorescens* C7R12¶	nosZ2F, nosZ2R, Henry *et al.* (2006)
amoA (AOA)	10	45 s at 50 °C	30	77–78	2×10^4	Fosmid clone 54d9**	19F, Leininger *et al.* (2006); CrenamoA 616r48x, Schauss *et al.* (2009)
amoA (AOB)	10	60 s at 60 °C	30	81–83	4.5×10^5	*Nitrosomonas europaea*	amoA-1F, amoA-2R, Rotthauwe *et al.* (1997)

*40 cycles started with 5 min initial denaturation and activation of polymerase at 95 °C.
†Efficiency was calculated using LINREGPCR (Ruijter *et al.*, 2009).
‡Touch down starting at 63 °C temperature decrease of 2 °C per cycle.
§Touch down starting at 63 °C temperature decrease of 1 °C per cycle.
¶Eparvier *et al.* (1991).
**Treusch *et al.* (2005).

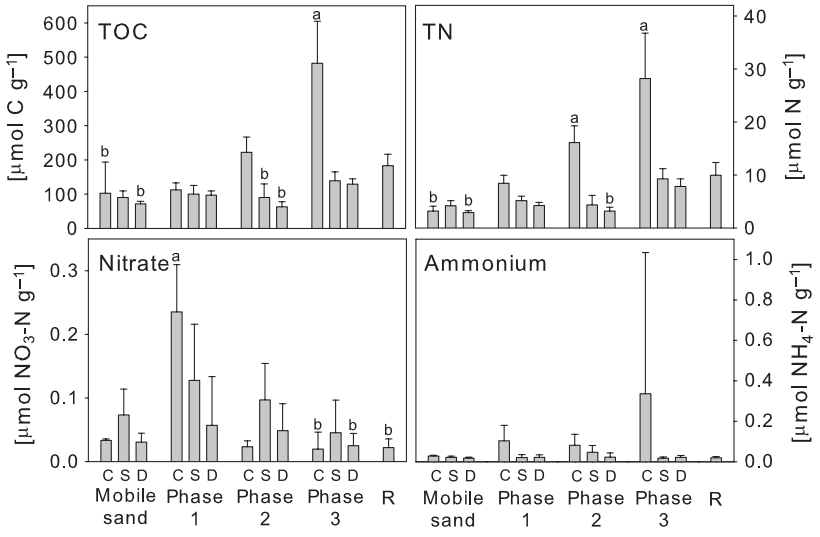

Fig. 2. TOC, TN, ammonium and nitrate. Bars are average of five replicates, and error bars indicate standard deviation. Significant differences tested by one-way ANOVA on ranks ($P < 0.05$) are indicated by different letters. Letters C, S and D indicate sampling depth, crust (0–5 mm depth), subsoil (5–15 mm depth) and deeper subsoil (15–35 mm depth), respectively.

The same pattern was observed in the subsoils: from mobile sand towards phase 3, TOC and TN content increased (Fig. 2). Content of TOC and TN in the reference (182 µmol C g^{-1}, 9.9 µmol N g^{-1}) was comparable with the subsoil of phase 3 (139 µmol C g^{-1}, 9.3 µmol N g^{-1}). In general, TOC and TN were decreasing in each of the phases from the crust to the deeper soils. Nitrate concentrations were highest in crust of phase 1 (0.24 µmol NO_3^--N g^{-1}) and did not follow any trend observed for the other chemical parameters (Fig. 2). Soil pH was around 4.8 in all samples and only slightly increased (not significant) in crust of phases 2 and 3, which had a pH of 5.2 (data not shown).

N pool sizes of TN, nitrate and ammonium were calculated for the total sampling depth of 35 mm (Fig. 3). The TN pool increased from 173 mmol N m^{-2} in mobile sand to 470 mmol N m^{-2} in phase 3. In the reference, the TN pool was 525 mmol N m^{-2}. The nitrate pool was

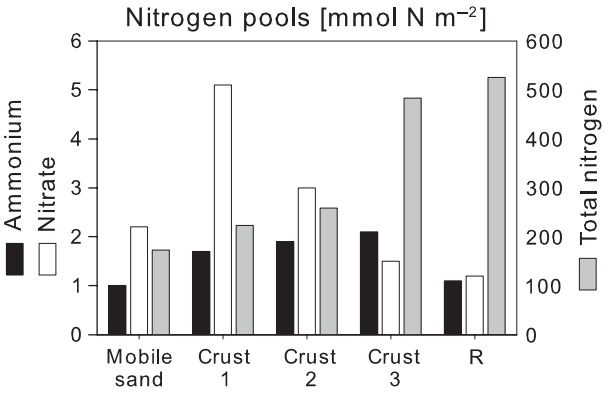

Fig. 3. TN, nitrate and ammonium pools for each phase of BSC development given in mmol N m^{-2}. For calculations, a soil depth of 35 mm was considered, and a bulk soil density of 1.5 g cm^{-3} was assumed. For mobile sand and phases 1 to 3, the 35 mm included crust (5 mm), subsoil (10 mm) and deeper subsoil (20 mm) samples.

the highest in phase 1 (5.1 mmol NO_3^--N m^{-2}) and lowest in phase 3 and reference. The ammonium pool was highest in phases 1, 2 and 3 (1.7–2.1 mmol NH_4^+-N m^{-2}) and slightly lower in mobile sand and the reference.

Development of BSCs

In addition to increasing nutrient content in the BSCs, crust repellency developed and chlorophyll content increased during BSC development (Fig. 4). Repellency index significantly increased from 0.9 in the mobile sand to 2.0 in phase 3. Chlorophyll content slightly increased (not significant) from mobile sand to phase 1 and phase 2. Towards phase 3, it significantly increased by one order of magnitude to 35 mg chlorophyll *a* m^{-2} and 12 mg chlorophyll *b* m^{-2}. The ratio between chlorophyll *a* and *b* decreased from 5.0 in phase 1 to 2.7 in phase 3, indicating a proportionally higher increase in chlorophyll *b*.

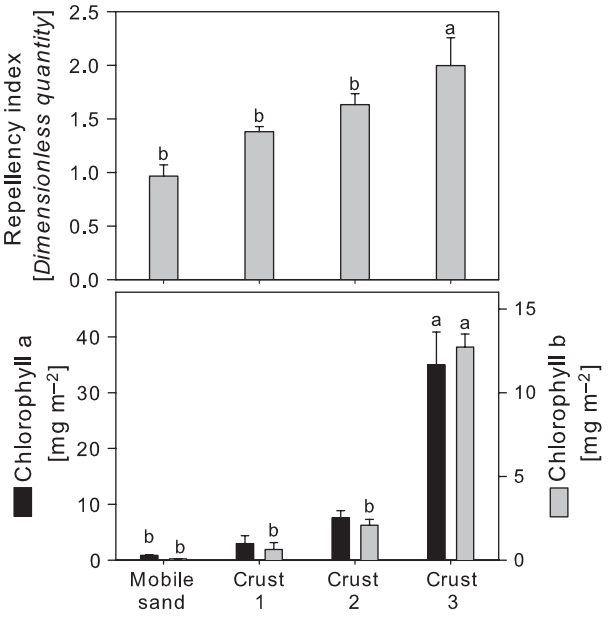

Fig. 4. Repellency index and chlorophyll contents of the BSCs. Significant differences tested by one-way ANOVA ($P < 0.05$) are indicated by different letters.

Potential enzyme activity

Within each different BSC phase, potential chitinase and denitrification activity decreased from the crust down to the subsoils, where activities were generally 2–10 times lower compared with the crusts (Fig. 5). When comparing the different phases with each other, a clear increase in potential enzyme activities was observed from phases 1–3. An increase in protease, nitrification and denitrification activity could first be observed in the subsoil and then in the deeper subsoil (Fig. 5). Activities in mobile sand and the deeper subsoil were often close to or below the LOD. In the reference sample, potential chitinase, protease and denitrification activities were similar to the activities observed in the subsoils of phase 3 and ranged between 0.1 and 0.3 nmol g^{-1} h^{-1}. In contrast, levels of potential nitrification activity in the reference sample (2.7 nmol NO_2^--N g^{-1} h^{-1}) were higher than in the subsoils and comparable to the crust of phase 3 (3.4 nmol NO_2^--N g^{-1} h^{-1}).

Gene abundance

All quantified functional genes were of lowest abundance in mobile sand and of highest abundance in phase 3 [*nifH*, *chiA*, *amoA* (AOA)] or the reference (*nosZ*) (Fig. 5). Two functional genes could not be quantified. The *aprA* gene was detected in all samples, but below the LOQ of 7×10^5 copies g^{-1}. The *amoA* gene of AOB was not detected; PCR on sample templates did not produce amplicons of the right size, but a number of unspecific bands and smear (gels not shown). For all functional genes quantified, the abundance was always higher in the crusts than in the subsoils. In contrast to enzyme activity, gene abundances did not show a steady increase from phase 1 to 3. While *nifH* and *chiA* abundance remained constant from phase 1 to phase 2, *amoA* (AOA) and *nosZ* abundance decreased in crust and subsoil of phase 2 and increased in phase 3 again. Abundance of *nosZ* was high in the crust of phase 1 (3.8×10^4 copies g^{-1}) and of similar range as in crust of phase 3 (3.5×10^4 copies g^{-1}). Ordination analysis of all soil parameters, potential

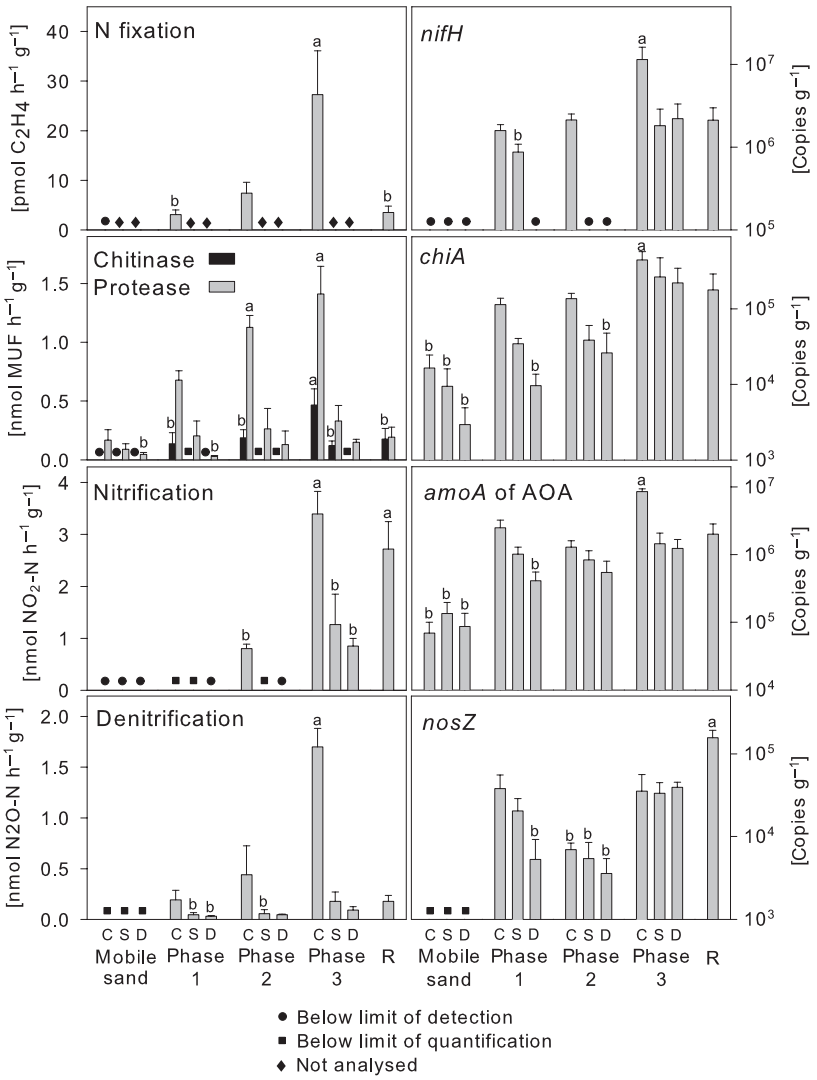

Fig. 5. Potential enzyme activities (left panel) and abundance of functional genes (right panel). Each bar represents mean of five replicates, error bars indicating the standard deviation. Significant differences tested by one-way ANOVA on ranks ($P < 0.05$) are indicated by different letters on top of the error bars. Letters C, S and D indicate sampling depth, crust (0–5 mm depth), subsoil (5–15 mm depth) and deeper subsoil (15–35 mm depth), respectively.

enzyme activities and gene abundance measurements did not discriminate BSC phases (data not shown).

Discussion

A prerequisite of our study was the presence of different phases of BSC development, and our measurements confirmed our first hypothesis that this was in fact the case. BSC development was evident from the distinct changes in chemical, physical and biological parameters (Belnap & Eldridge, 2001; Fischer *et al.*, 2010). As expected, the development proceeded from mobile sand, via BSC of phase 1 and 2, to the established BSC in phase 3. In this order, TOC, TN and chlorophyll content increased, and measurements in phase 2 and 3 are comparable to previous characterisations of BSCs in arid (Barger *et al.*, 2005; Johnson *et al.*, 2005) and temperate climate (Langhans *et al.*, 2009; Fischer *et al.*, 2010). The proportional decrease in chlorophyll *a* compared with chlorophyll *b* indicates a shift from cyanobacterial BSCs to BSCs dominated by algae and mosses (Belnap & Eldridge, 2001; Zaady *et al.*, 2010), confirming that our sampling approach represents typical BSC development. Langhans *et al.* (2009) found significant differences in floral composition between initial and developed BSC in temperate climate; however, TN content did not differ. In contrast, we identified changes in TN content and other parameters, underlining the presence of a gradient of BSC development at the Lieberose dune.

Beyond the changes in chemical, physical and biological parameters that occur during BSC development, we were interested in the development of the N cycling processes that depend on N availability. While TN concentrations increased in the crusts and in the subsoils,

they were always higher in the subsoils right under the crust compared with the deeper subsoil, indicating that a translocation from the crusts to the underlying soil took place (Johnson *et al.*, 2005). The pool size of TN showed a strong increase from mobile sand to phase 3, and TN pool in the reference was comparable to that of crust 3. A previous study on BSC of temperate climate found similar levels of TN, nitrate and ammonium (Langhans *et al.*, 2009). This might indicate that TN reaches a steady state in the BSC system (Wallace *et al.*, 1978). Nitrate and ammonium content did not follow the pattern of nutrient increase. The highest nitrate concentrations and nitrate pools were found in phase 1, while the highest ammonium concentrations and ammonium pools were found in phase 3. During phase 1, the BSC is thin and oxygen penetration is high, and this might favour the process of nitrification while denitrification rate is low, causing an accumulation of nitrate. In contrast, in the developed crust of phase 3, TOC content is high and anaerobic conditions may form after rewetting events, facilitating denitrification (Johnson *et al.*, 2007) and causing depletion in nitrate. This is supported by the high potential denitrification rates measured in phase 3. The ammonium might originate from N fixation, as the development of N fixation parallels ammonium concentrations and has been reported to increase soil ammonium content (Klubek *et al.*, 1978; Evans & Belnap, 1999).

In addition to the N pool sizes, the redistribution of N in the soil will impact N availability, thus affecting N cycling processes. The soil hydrology, in particular, affects N redistribution in sand dunes, as was previously shown for arid ecosystems (Evans & Lange, 2001; Yair, 2001; Johnson *et al.*, 2007). At the studied dune, the slope is gentle, and BSC repellency is low in the upper part of the slope. Therefore, we expect infiltration and vertical N transport rather than surface run-off and lateral N allocation. The evaporation length in this soil was estimated to be 25 cm depth (D. Or, pers. commun.), that is, the depth water can be drawn back to the surface by capillary force. For this reason, all dissolved nutrients leaching below 25 cm depth become inaccessible for the BSCs. Heavy rains are expected to leach dissolved nutrients. However, mild to moderate rain may not move nutrients beyond the 25-cm depth. This means that during moderate rains, N would not be lost via leaching, but would continue to accumulate in the dune. For this reason, we modelled the water flux at the dune surface and at a depth of 25 cm depth for the year 2010. We could identify a number of rain events that caused a water flux at the surface, but did not lead to water flux at 25 cm depth (Fig. 1). If we assume that the rain event, which occurred 10 days prior to our sampling (18 mm), leached all nitrate and ammonium, then 2.9 mmol N m^{-2} would have been deposited (atmospheric N deposition rate of 12 μmol m^{-2} h^{-1}) at our site within the 10-day period between the rain event and our sampling. This is in the same order of magnitude as the nitrate and ammonium pools we could measure.

To assess N turnover of each phase of BSC development, we calculated area-based enzyme activities (Fig. 6). We are aware that these calculations are tentative, as they rely on potential enzyme activities only. Additionally, the different enzyme assays vary in methodology and are not comparable. Nevertheless, we find this synopsis useful and stimulating for interpreting our results.

The development of the N cycling processes proceeded in a distinct order. This confirmed our second hypothesis, and we propose three stages of N cycle succession. First, *the heterotrophic stage*, represented by the mobile sand; second, *the transition stage*, which is represented by phase 1 and phase 2; third, *the developed stage*, represented by phase 3 and reference.

The heterotrophic stage (mobile sand) is characterised by mineralisation as the prevailing process and the

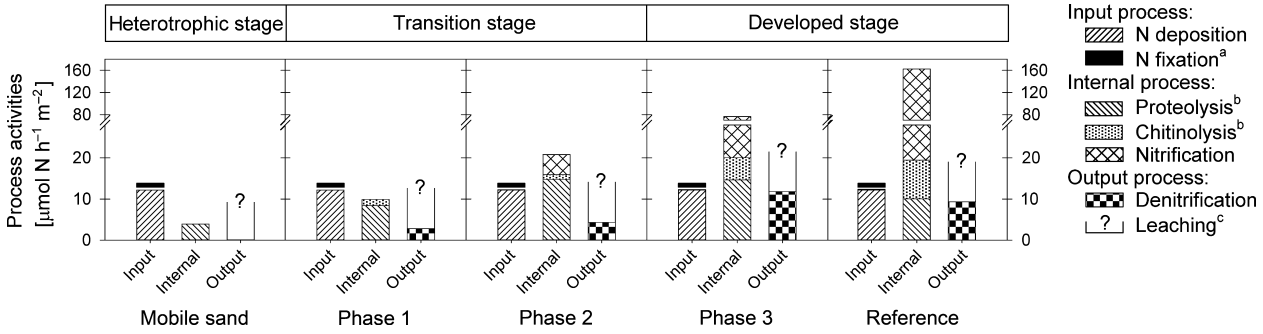

Fig. 6. Potential process activities for each phase of BSC development. Each diagram gives input, output and internal turnover terms as determined for each phase of BSC development. Processes were calculated for considering 35 mm soil depth and assuming a bulk soil density of 1.5 g cm^{-3}. For mobile sand and phases 1–3, the 35 mm included crust (5 mm), subsoil (10 mm) and deeper subsoil (20 mm) samples.

dominance of opportunistic heterotrophic bacteria. The heterotrophic stage was described earlier in glacier forefields (Sigler *et al.*, 2002; Brankatschk *et al.*, 2011), and Hodkinson *et al.* (2002) proposed an initial heterotrophic stage as general rule in primary succession. In mobile sand, we found *aprA* and *chiA* genes, and protease activity was present in the same order of magnitude as previously reported mineralisation rates in the Negev Desert (Zaady, 1996). In the heterotrophic stage, cyanobacteria and algae are virtually absent. Therefore, the carbon sources of the microbial community may be allochthonous (Hodkinson *et al.*, 2002; Brankatschk *et al.*, 2011) and/or recalcitrant (Bardgett *et al.*, 2007) organic matter. Quantifying the macroscopic litter (> 2 mm) in mobile sand, we found 0.2 g m^{-2} of litter material, such as birch leaves, birch seeds and tree bark (data not shown). This indicates that approximately 3 % of TOC in mobile sand is allochthonous and readily available carbon. By metabolising the organic matter, the heterotrophic microorganisms probably release inorganic N to the soil. This N, together with the inorganic N from atmospheric deposition, is easily leached, and N does not accumulate during that stage.

In *the transition stage* (phase 1 and phase 2), cyanobacteria and algae establish in the BSC, and—via photosynthesis—build up organic matter, which requires a steady N supply. Although N fixation activity and *nifH* gene abundance increase, the contribution of N fixation to soil N supply appears to be low. Atmospheric deposition rates are two orders of magnitude higher than N fixation activity (0.1 μmol C_2H_4 m^{-2} h^{-1}) ranging from 9.8 to 12.2 μmol N m^{-2} h^{-1} (12–15 kg N ha^{-1} a^{-1}) (Gauger *et al.*, 2008). Previously reported N fixation rates in BSCs ranged from 50 to 100 μmol C_2H_4 m^{-2} h^{-1} (MacGregor & Johnson, 1971; Jeffries *et al.*, 1992; Abed *et al.*, 2010) down to 0.02 μmol C_2H_4 m^{-2} h^{-1} (Evans & Belnap, 1999; Strauss *et al.*, 2011). Despite the fact that *nifH* gene abundance at the Lieberose sand dune is the same as in the Colorado Plateau and the Chihuahuan Desert, USA (Yeager *et al.*, 2004, 2007), we found a comparatively low N fixation activity. This difference is likely due to the early developing phase (Barger *et al.*, 2005) and high N deposition rates (Vitousek *et al.*, 2002) that are typical for Central Europe. The discrepancy between gene abundance and potential enzyme activity might be due to different levels of cell activity (Röling, 2007). Furthermore, a given process may be catalysed by a broader variety of enzymes, whereas the primers used in the study may only capture a selected number of microorganisms. In the transition stage, the potential denitrification activity increases, ranging from 2.8 to 4.3 μmol N m^{-2} h^{-1}, which is comparable to previous studies (Peterjohn & Schlesinger, 1991). Conditions for denitrification might be ideal during the transition stage, as both important substrates for denitrification, that is, nitrate and carbon (Zumft, 1997), are available. The atmospheric deposition rate of nitrate is high, and cyanobacteria excrete extracellular polymeric substances that are readily available carbon sources (Mazor *et al.*, 1996).

In *the developed stage* (phase 3 and reference), the potential enzyme activities are comparable to developed ecosystems (Peterjohn & Schlesinger, 1991; Johnson *et al.*, 2007; Brankatschk *et al.*, 2011) and might advance towards a steady state. The N cycle is dominated by the nitrification process (Fig. 6) and marks the shift towards internal N cycling. Nitrification rates in the crust of phase 3 were comparable to rates measured in the Great Basin, USA (Skujins *et al.*, 1978). The nitrification activity pattern is matched by gene abundance, showing highest *amoA* (AOA) gene abundance in crusts. Interestingly, *amoA* gene abundance of AOB was below 1200 copies per g soil, that is, two to three orders of magnitude lower than AOA abundance. This is supported by studies reporting that AOA outnumber AOB up to 200-fold (Leininger *et al.*, 2006) and suggests that AOA show a greater relative activity compared with AOB, as was previously reported from other acidic environments (Nicol *et al.*, 2008).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Image of mobile sand.
Fig. S2. Image of crust 1.
Fig. S3. Image of crust 2.
Fig. S4. Image of crust 3.
Fig. S5. Image of *Corynephorus canescens*.

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