

# A natural $^{15}\text{N}$ approach to determine the biological fixation of atmospheric nitrogen by biological soil crusts of the Negev Desert

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Biological soil crusts are important cryptogamic communities covering the sand dunes of the north-western Negev. The biological crusts contain cyanobacteria and other free-living  $\text{N}_2$ -fixing bacteria and are hence able to fix atmospheric nitrogen (N). This is why they are considered to be one of the main N input pathways into the desert ecosystem. However, up to now, *in situ* determinations of the  $\text{N}_2$  fixation in the field are not known to have been carried out. We examined the natural  $^{15}\text{N}$  method to determine the biological  $\text{N}_2$  fixation by these soil crusts under field conditions. This novel natural  $^{15}\text{N}$  method uses the lichen *Squamarina* with symbiotic green algae—which are unable to fix  $\text{N}_2$ —as a reference in order to determine  $\text{N}_2$  fixation. Depending on the sampling location and year, the relative biological fixation of atmospheric nitrogen was estimated at 84–91% of the total N content of the biological soil crust. The cyanobacteria-containing soil lichen *Collema* had a fixation rate of about 88%. These fixation rates were used to derive an absolute atmospheric N input of 10–41 kg N ha<sup>-1</sup> year<sup>-1</sup>. These values are reasonable results for the fixation of atmospheric  $\text{N}_2$  by the biological crusts and cyanolichens and are in agreement with other comparable lab investigations. As far as we are aware, the results presented are the first to have been obtained from *in situ* field measurements, albeit only one location of the Negev with a small number of samples was investigated. Copyright © 2005 John Wiley & Sons, Ltd.

In addition to the availability of water, nitrogen (N) controls the primary production and most other biological activities in desert ecosystems.<sup>1,2</sup> The biological fixation of atmospheric nitrogen by cyanobacteria in biological soil crusts and lichens is considered—besides that of free-living  $\text{N}_2$ -fixing bacteria in C-rich macrophytic patches—to be a main N input pathway into dryland ecosystems.<sup>3–11</sup> Soil crusts have been found in locations such as arid and semi-arid areas all over the world.<sup>3–5,7,10,12–14</sup> Large areas of the sand dunes of the north-western Negev are also covered by these soil crusts. The importance of biological  $\text{N}_2$  fixation (BNF) by soil crusts has been emphasised by several authors (e.g. Refs. 3–9), but the determination of the N fixation under field conditions has many methodological problems.<sup>5,7,11</sup> Most of the information about the contribution to the N input in different dry ecosystems only originates from lab investigations or simple estimates based on crust development and total N content. In lab investigations, acetylene reduction and  $^{15}\text{N}_2$  incubation assays under simulated field conditions have been performed.<sup>4,7,14–16</sup> The estimation of the annual N

inputs at field scale by BNF of biological crusts and cyanobacterial lichens using a combination of lab investigations and actual weather conditions including surface moisture is somewhat unreliable.<sup>11</sup> As a result, a wide variation has been revealed among different drylands investigated under simulated field conditions.<sup>1,4,5,7,11</sup> In general, one can conclude that the annual N inputs into the dryland's ecosystem by BNF of biological crusts and cyanobacterial lichens amount to 1–10 g N m<sup>-2</sup> year<sup>-1</sup>. Measurements of N fixation by cyanobacteria in the biological crust of the Negev Desert are unknown apart from a laboratory study using the acetylene reduction assay.<sup>7</sup>

A common way to determine the BNF of legume-*Rhizobium* symbioses is the natural  $^{15}\text{N}$  abundance method.<sup>17–21</sup> The advantage of this method is that it can be easily applied at any field site without the need for additional  $^{15}\text{N}$  labelling (for explanation of the method, see below). However, as far as cyanobacteria in biological crusts and lichens are concerned, no publications dealing with the natural  $^{15}\text{N}$  abundance to determine quantitatively BNF are known up to the present.

In this paper we present a novel approach of the natural  $^{15}\text{N}$  abundance technique by utilising the non- $\text{N}_2$ -fixing lichens *Squamarina lentigeria* and *S. crassa* as a reference in order to determine  $\text{N}_2$  fixation by the biological crust and cyanobacteria lichens *in situ* in the Negev Desert.

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## EXPERIMENTAL

### Study site

The study was carried out in the sand dunes of the north-western Negev, which is situated in the most eastern part of the sand field covering the northern part of the Sinai Peninsula.<sup>22</sup> Three sampling locations (SL) were sampled.<sup>23,24</sup> In the current study only the 'Haluza sand field' (SL 3, also named Nizzana North) could be considered, as both soil crusts and lichens with cyanobacteria and green algae as a photobiont were present (for situation of SL 3, see map in Refs. 23 or 24). The climate of the Negev is characterised by a sharp gradient from the north to the arid south and the average annual rainfall decreases from around 170 mm at the northern edge of the sand field near Yevul to approximately 90 mm near Nizzana.<sup>24,25</sup> The rainfall is limited to the winter season (October to March). The mean annual temperature at the Nizzana experimental site is around 20°C (mean minimum: 12.5°C; mean maximum: 26.5°C). At the sampling location in the years investigated the current rainfall was as follows: winter 1997/98: 30.3 mm; winter 1998/99: 53.1 mm.

### Biological soil crusts

The crusts cover the uppermost few millimetres of the soil surface and are built up by green algae, cyanobacteria and local mosses and soil crust lichens.<sup>9,13,26</sup> Nitrogen-fixing cyanobacteria such as *Nostoc* spp., *Scytonema* spp., *Phormidium* spp., *Oscillatoria* spp., and heterotrophic free-living N<sub>2</sub>-fixing bacteria, are common in these types of soil crusts.<sup>27</sup> Soil lichens with cyanobacterial phytobionts are also able to fix N. The cover of the biological crusts on the sand dune estimated from satellite images amounts to approximately 68% in Haluza (SL 3). The soil crust cover of the interdune ranges between 92–97%, whereas the cover on the dune crest is only about 29%.<sup>9,24</sup> The thickness of the crusts ranged between 1–7 mm depending on the dune exposition. This lichen community is composed of *Fulgensia fulgens* (Teloschistaceae), *Squamarina cartilaginea*, *S. crassa* (Lecanoraceae), *S. lentigera* and *Diploschistes diacapsis*; syn. *D. steppicus* (Diploschistaceae). N<sub>2</sub>-fixating soil lichens are *Collema* spp. (Collemaaceae) and other non-identified cyanobacterial lichens. In the interdunes, the cyanobacterial lichens dominate the soil crusts. Locally, *Fulgensia fulgens* could be found on north-west facing slopes and interdunes, where *Fulgensia* covered between 15–85% of the area. The lichen cover of the genus *Squamarina* in this locality ranged between 5–8%.

### Sampling

Samples of biological crusts, the underlying sand down to a depth of 90 cm and lichens (*Collema* spp., *Fulgensia fulgens*, *Squamarina lentigera* and *S. crassa*) were taken in March 1998 and 1999 at SL 3 'Haluza sand field'. Samples of the crust and soil were taken in three replicates from two different plots of the location. The material was oven-dried at 105°C and ground to a fine homogeneous powder for <sup>15</sup>N measurement.

### <sup>15</sup>N methodology and calculation of biological N fixation

The <sup>15</sup>N abundance in the pre-treated samples was measured on an DELTA S isotope mass spectrometer (Finnigan MAT

Bremen, Germany) coupled online with an elemental analyser CHN EA 1108 (Carlo Erba, Italy) (for details, see Ref. 23). The natural <sup>15</sup>N abundances measured are given in δ‰ units.<sup>28</sup>

The <sup>15</sup>N method to determine the biological N<sub>2</sub> fixation of legume-*Rhizobium* symbioses introduced by McAuliffe *et al.*<sup>29</sup> was originally a <sup>15</sup>N isotope dilution technique by the artificial tracing of the plant-available N pool with <sup>15</sup>N. The different variations of this technique are explained in Russow and Faust.<sup>30</sup> The methodical variations of the natural <sup>15</sup>N abundance technique use the δ<sup>15</sup>N enrichment of the soil N in comparison to N derived from the atmosphere (NdfA) as a natural <sup>15</sup>N tracer.

Russow *et al.*<sup>23</sup> observed that the δ<sup>15</sup>N values of the biological soil crusts and the cyanobacteria lichens *Collema tenax* spp. are all close to zero (down to –2‰) in the study site. In contrast, the non-N-fixing soil lichens of the genus *Squamarina* showed a very low δ<sup>15</sup>N of average –11‰. This very negative value of *Squamarina*, in comparison to the N<sub>2</sub>-fixing crusts and lichens, we used as a natural <sup>15</sup>N tracer to estimate the BNF of the soil crusts in the Haluza sands, i.e. we used *Squamarina* as a reference in the sense of the natural <sup>15</sup>N variation method.

The NdfA can now be calculated using a two-pool model from the quotient of the natural <sup>15</sup>N abundance of the N<sub>2</sub>-fixing cyanobacteria (in crusts or lichens) and the non-N<sub>2</sub>-fixing *Squamarina* (Eqn. (1)).

$$\text{NdfA} = (\delta_n - \delta_f / \delta_n - \delta_b) \times 100 \quad (1)$$

where δ<sub>f</sub> = δ<sup>15</sup>N of N<sub>2</sub>-fixing cyanobacteria in ‰; δ<sub>n</sub> = δ<sup>15</sup>N of non-N<sub>2</sub>-fixing lichens in ‰; and δ<sub>b</sub> = isotopic shift against the δ<sup>15</sup>N signature of air by BNF itself

BNF is known to create a slight isotopic <sup>15</sup>N shift down to about –2‰ against the δ<sup>15</sup>N signature of air (0‰) by a kinetic isotope effect,<sup>20</sup> but this shift is not known for cyanobacteria. Therefore, to avoid an overestimation of the BNF of the crust, here the δ<sub>b</sub> was set to ±0‰. Thus, Eqn. (1) becomes Eqn. (2).

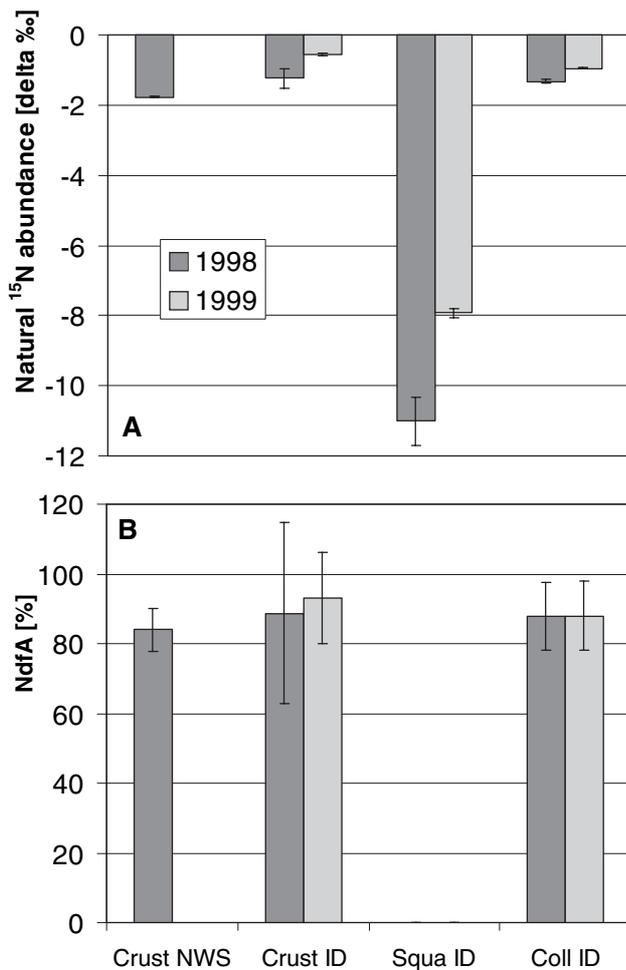
$$\text{NdfA} = (\delta_n - \delta_f / \delta_n) \times 100 \quad (2)$$

More details of this novel <sup>15</sup>N approach for the *in situ* determination of the BNF of soil crusts and cyanobacteria lichens are discussed below.

## RESULTS

The δ<sup>15</sup>N values of the biological soil crusts and lichens in the Haluza sands (SL 3) are all negative, but differ significantly among the various species sampled (Fig. 1(A)).

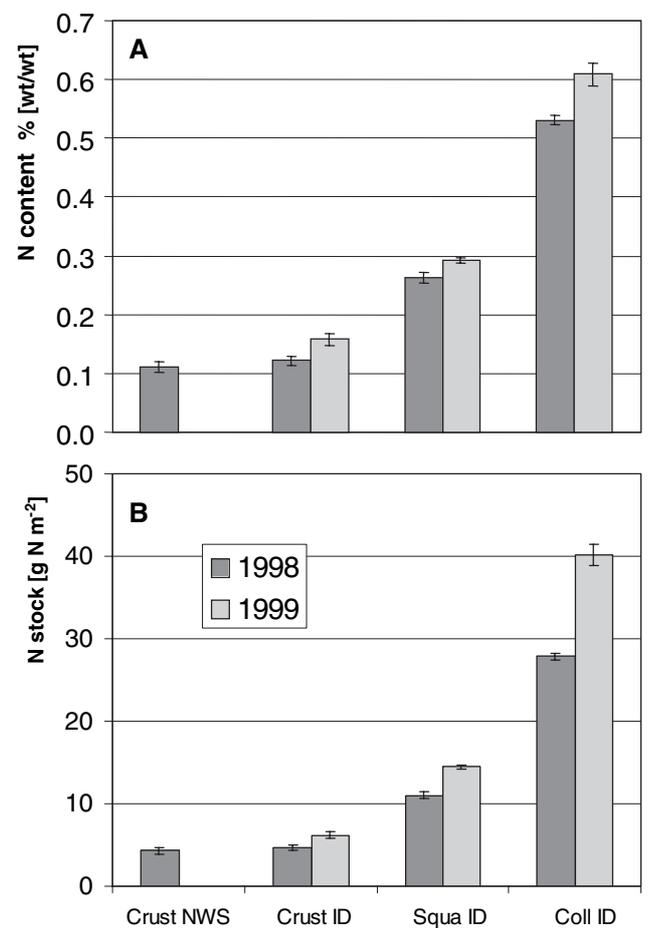
The thickness and density of the crusts and lichens sampled are listed in Table 1. These values and the N content of the samples (Fig. 2(A)) can be used to calculate the stock of N, which is shown in Fig. 2(B). As shown, the biological crusts and soil lichens contain considerable stocks of N which rise with increasing thickness from 5–7 g N m<sup>-2</sup> in cyanobacterial crusts to 28–40 g N m<sup>-2</sup> in the soil lichen crusts. The thickness of the crusts varies greatly from one location to the next—from 1 to 4 mm for biological soil crusts and from 4 to 7 mm for soil lichens. The amounts of N calculated (Fig. 2(B)) are therefore only valid for the particular sampling spot in question and generally exhibit wide variation. The



**Figure 1.** Natural  $^{15}\text{N}$  abundance given in  $\delta^{15}\text{N}\text{‰}$  (A) and relative biological  $\text{N}_2$  fixation (Ndfa) (B) for soil crusts and lichens at Haluza sands (SL 3). Crust NWS—biological crust on north-west-facing slope; Crust ID—biological crust on interdune; Squa ID—*Squamarina* spp. on interdune; Coll ID—*Collema* spp. at interdune.

values of BNF of the biological soil crusts and crustal lichens calculated as relative Ndfa values according to Eqn. (2) are shown in Fig. 1(B).

The Ndfa only provides relative values to the BNF and does not reflect the absolute  $\text{N}$  input into the ecosystem. The  $\text{N}$  input can only be calculated by additionally using the  $\text{N}$  stock ( $m$ ) and the annual growth rates of the pool considered. As far as crusts are concerned, no growth data for undisturbed crusts are available. We therefore commenced by using the regeneration time ( $\tau$ ) for the soil crust from an experiment in which large areas of the crust in the interdunes at the Nizzana test site were completely removed and the increase in chlorophyll content depending on the time after



**Figure 2.** N content (A) and N stock (B) for biological crusts and lichens at Haluza sands (SL 3).

removal was measured.<sup>9</sup> The regeneration time  $\tau$  could be approximately estimated according to a logarithmic approach for soil crust (3 mm) to 3–4 years and for lichen crust (4–5 mm) to 7–8 years. The calculation of the absolute  $\text{N}$  input by cyanobacteria is carried out according to Eqn. (3):

$$\text{BNF} = \text{Ndfa} \times m / \tau \quad (3)$$

The average Ndfa values and  $\text{N}$  stocks were taken from Figs. 1(B) and 2(B) and the absolute biological  $\text{N}_2$  fixation calculated from these values is shown in Table 1. This ranges from 1.0 to 1.2  $\text{g N m}^{-2} \text{ year}^{-1}$  for cyanobacterial crusts and up to 4.1  $\text{g N m}^{-2} \text{ year}^{-1}$  for *Collema tenax* spp.

## DISCUSSION

As already mentioned in the introduction, measurements of  $\text{N}$  fixation by cyanobacteria in the biological soil crusts from

**Table 1.** Thickness ( $x$ ), density ( $\rho$ ), regeneration time ( $\tau$ ) and absolute biological  $\text{N}$  fixation (BNF) of biological crust types at different locations within the Haluza sands (SL 3, ID—interdune; NWS—north-west-facing slope)

Species (location)	Remarks	$x$ [mm]	$\rho$ [ $\text{g cm}^{-3}$ ]	$\tau$ [year]	Abs. BNF [ $\text{g N m}^{-2} \text{ year}^{-1}$ ]
<i>Squamarina</i> spp. (ID)	Photobiont: green algae	4.0	1.05	5.5	0
<i>Collema tenax</i> spp. (ID)	Photobiont: cyanobacteria	5.0	1.05	7.6	4.1
Biological soil crust (ID)	Cyanobacteria and green algae	3.0	1.3	4	1.2
Biological soil crust (NWS)	Cyanobacteria and green algae	3.0	1.3	3.5	1.0

the Negev Desert are unknown, with the exception of laboratory studies using the acetylene reduction assay.<sup>7</sup> Our results with the natural <sup>15</sup>N abundance method showed clearly that biological crusts are able to fix a significant amount of N. Measuring the natural <sup>15</sup>N abundance of the non-fixing crustal lichens *Squamarina lentigera* and *S. crassa* in comparison to the cyanobacterial soil crusts and cyanolichens enables the estimation of the fixation under field conditions. This novel approach for the *in situ* determination of N<sub>2</sub> fixation by biological crusts in the field is explained as follows. Assuming that no soil N moves upwards from the soil into the crust, the crusts obtain their nitrogen solely from two N pools:

- the atmospheric N<sub>2</sub> fixed by the cyanobacteria present in the biological crust (BNF);
- airborne N deposition.

The lichens *Squamarina lentigera* and *S. crassa*—which do not contain cyanobacteria—cannot fix N<sub>2</sub> and consequently obtain their N solely from airborne N deposition. This assumption is supported by other investigations in which lichens were used to monitor air pollution.<sup>31–33</sup> As shown in Fig. 1(A), the  $\delta^{15}\text{N}$  of these non-N<sub>2</sub>-fixing lichens is extremely negative down to an average of  $-11\%$ , probably caused by the absorption of strongly negative airborne N in either gaseous form (ammonia, NO<sub>x</sub>) or from rainwater uptake (ammonium/ammonium, nitrate).<sup>34–36</sup> Although natural <sup>15</sup>N figures for lichens in arid regions are unknown, measurements in other regions support our assumption. The  $\delta^{15}\text{N}$  values of the lichens *Hypogymnia physodes* and *Pseudevernia furfuracea* from the eastern central Alps range from  $-4$  to  $-7\%$ , depending on altitude,<sup>37</sup> even attaining  $-14\%$  for *H. physodes* in a forest in central Germany (K. Jung, personal communication). For epiphytic lichens, Comstock<sup>38</sup> found values of between  $-4$  and  $-8\%$  in the Kings Canyon and Glacier National Parks (USA) and Franzen-Reut<sup>33</sup> values down to  $-11\%$  at different sites with anthropogenic impacts of the German state North Rhine-Westphalia.

The natural <sup>15</sup>N abundance technique should also be applicable to determine the fixation of N<sub>2</sub> from the atmosphere by biological crusts and cyanobacteria-containing lichens, because of the relatively large difference between the  $\delta^{15}\text{N}$  value of the atmospheric N pool (0‰) and the non-N<sub>2</sub>-fixing lichen *Squamarina* ( $-11\%$ ). The negative <sup>15</sup>N figure of the airborne N deposition is used as an N tracer and the non-N<sub>2</sub>-fixing lichen as a reference for this negative airborne <sup>15</sup>N. We have no exact proof that *Squamarina* is not contaminated by free-living N<sub>2</sub>-fixing bacteria and therefore does not fix N<sub>2</sub>. However, as its  $\delta^{15}\text{N}$  is very negative, there is evidently no or only negligible contamination by N<sub>2</sub>-fixing bacteria, and thus this species can be used as a reference for the <sup>15</sup>N abundance of airborne N deposition.

The precision of this approach depends primarily on the difference between the <sup>15</sup>N in the fixing and non-fixing lichens. Furthermore, as in all natural <sup>15</sup>N methods, there can be certain limitations caused by isotopic effects.<sup>19,21,23,39</sup> This means that estimates based on the natural <sup>15</sup>N abundance method are often only semi-quantitative.<sup>21</sup>

The calculated relative contribution of atmospheric N<sub>2</sub> (NdfA) by cyanobacteria of the biological crusts to the total N

content is extremely high at 84–91%. The investigated cyanolichens *Collema* spp. have a similar fixation rate (88% NdfA).

Evans and Lange<sup>12</sup> had already tried to utilise the natural <sup>15</sup>N abundance to estimate the relative contribution of the N<sub>2</sub> fixation (NdfA) to the total N in biological crusts. They used the natural <sup>15</sup>N values reported by Evans and Belnap<sup>40</sup> (crusts from throughout North America, Africa and Australia) and Evans and Ehleringer<sup>6</sup> (southern Utah, USA). Evans and Lange<sup>12</sup> found a strong negative correlation between the  $\delta^{15}\text{N}$  of the soil crust and nitrogenase activity of the crusts with  $\delta^{15}\text{N}$  values close to 0‰ (atmospheric N<sub>2</sub>) in the case of highest nitrogenase activity, i.e. highest BNF. Probably, this negative correlation is caused by an influence of soil N to the crusts (soil N having mostly positive  $\delta^{15}\text{N}$  values) or atmospheric bulk deposition with positive  $\delta^{15}\text{N}$  values. The authors compared the  $\delta^{15}\text{N}$  values of the crust with that of soil in order to determine the NdfA of the soil crust. However, soil is not suitable as reference and thus they could only qualitatively estimate the NdfA.

The relative fixation rate expressed as NdfA calculated above does not reflect the absolute N input into the ecosystem. For the calculation of the absolute N input in, for example, g N m<sup>-2</sup> year<sup>-1</sup>, the annual growth rates of the lichens and crusts are needed, respectively. The problem is that no growth data for undisturbed crusts and lichens are available. As explained in the Results section, we attempted to solve this problem by using the regeneration time  $\tau$  estimated from an experiment in which large areas of the crust were completely removed and the degree of restoration depending on the time after removal was measured. It is surely true that under steady-state conditions the growth rates of the lichens and crusts are lower, i.e. the absolute N input calculated hereafter is the maximum rate. Taking into account this restriction, the estimated absolute biological N<sub>2</sub> fixation amounts to between 1.0 and 1.2 g m<sup>-2</sup> year<sup>-1</sup> for cyanobacterial crusts and up to 4.1 g m<sup>-2</sup> year<sup>-1</sup> for *Collema* spp.; therefore within the range of 1–10 g m<sup>-2</sup> year<sup>-1</sup> published by other authors.<sup>1,4,5,41</sup>

Under optimum light and moisture conditions in a lab experiment, Zaady *et al.*<sup>7</sup> determined an N fixation rate of max. 34 nmol N cm<sup>-2</sup> h<sup>-1</sup> (=9.5 mg N m<sup>-2</sup> h<sup>-1</sup>) for biological crusts from the Negev through acetylene reduction. From our own measurements of the activity of biological crusts depending on dewfall and rain, an average effective fixation period following a dew or rain event of 45 and 150 min, respectively, can be concluded.<sup>9</sup> Taking into account an average of 195 days with sufficient dewfall for the study site and 15 rainy days per year,<sup>25</sup> this produces approximately 190 h year<sup>-1</sup> with almost optimum fixation conditions. This effective fixation time would enable a total fixation of 1.8 g N m<sup>-2</sup> year<sup>-1</sup> by the crusts, based on the above potential fixation rate of 9.5 mg N m<sup>-2</sup> h<sup>-1</sup>. The results of our field measurements (1.0–1.2 g m<sup>-2</sup> year<sup>-1</sup>) amount to 55–67% of this estimated potential N fixation by biological crusts (without *Collema* spp.).

In a recent publication, Belnap<sup>11</sup> reported an annual N input by BNF of 9 and 13 kg N ha<sup>-1</sup> year<sup>-1</sup> for dark crusts and crusts with 20% cover of *Collema tenax*, respectively, in the Canyonlands National Park, south-eastern Utah, USA. These

values were estimated from the results of acetylene reduction measurements in the lab in a similar approach to our own methods described above with the lab measurements of Zaady *et al.*<sup>7</sup> This approach has two primary uncertainties:

1. the wide variation in the factor for the conversion of the acetylene values to real  $\text{N}_2$  fixation. Belnap<sup>11</sup> and Liengen<sup>42</sup> reported for this factor a range of between 0.02 and 0.40;
2. the extrapolation from this lab value obtained under optimum fixation conditions to the  $\text{N}_2$  fixation in the field, taking into account the real temperature, precipitation and moisture of the crusts measured in the field for the observation period chosen.

In our *in situ* field measurements at the Haluza sands (SL 3), the average cover of the soil crusts was 68% with a share of about 20% *Collema* lichens. Under these circumstances, we determined an N input of about  $11 \text{ kg N ha}^{-1} \text{ year}^{-1}$ , a value which is fairly close to Belnap's result.<sup>11</sup>

These results show that the BNF by cyanolichens and biological soil crusts are very important N input pathways into the desert ecosystem of the Negev while N input by dust deposition can be considered as a minor pathway at just  $2\text{--}4 \text{ kg N ha}^{-1} \text{ year}^{-1}$ .<sup>23,43</sup>

A comparison of the results from this study with other *in situ* field measurements is not possible due to the lack of relevant results in the literature. However, the comparison with the results of the lab-assisted estimation of the  $\text{N}_2$  fixation in the field, discussed above, and other lab investigations under simulated field conditions—e.g. in the Great Basin<sup>4,5,41</sup> ranging from  $1\text{--}10 \text{ g N m}^{-2} \text{ year}^{-1}$ —demonstrate that the examined natural  $^{15}\text{N}$  method gives reasonable values for the  $\text{N}_2$  fixation by biological soil crusts and cyanobacteria-containing soil lichens.

## CONCLUSIONS

From our field investigations, we conclude that the novel approach utilising the non-fixing lichen *Squamarina* as a reference for the natural  $^{15}\text{N}$  abundance method results in reasonable estimated values for the fixation of atmospheric  $\text{N}_2$  by biological soil crusts and cyanobacteria-containing soil lichens.

This study demonstrates that biological soil crusts contribute significant amounts of fixed atmospheric N to the Negev Desert ecosystem. The relative  $\text{N}_2$  fixation rates (NdfA) by cyanobacteria of the biological crusts and cyanolichens *Collema* spp. are extremely high at 84–91%.

The absolute N input by cyanobacteria into the ecosystem calculated from the NdfA values is slightly unreliable as no precise annual growth rates of the lichens and crusts are available. Nonetheless, the estimated absolute biological  $\text{N}_2$  fixation by biological crusts and *Collema* lichens of  $1.0\text{--}1.2 \text{ g N m}^{-2} \text{ year}^{-1}$  ( $10\text{--}12 \text{ kg N ha}^{-1} \text{ year}^{-1}$ ) and of about  $4.1 \text{ g N m}^{-2} \text{ year}^{-1}$  ( $41 \text{ kg N ha}^{-1} \text{ year}^{-1}$ ), respectively, represent significant N input pathways. The extrapolation of these values for the total area of 'Haluza' by taking into account the crust soil cover results in an N input of about  $11 \text{ kg N ha}^{-1} \text{ year}^{-1}$ .

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