

USING THE NATURAL ^{15}N ABUNDANCE TO ASSESS THE MAIN NITROGEN INPUTS INTO THE SAND DUNE AREA OF THE NORTH-WESTERN NEGEV DESERT (ISRAEL)

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Dedicated to Prof. Dr. Dr. h.c. Peter Fritz on his retirement.

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The variation of the natural ^{15}N abundance is often used to evaluate the origin of nitrogen or the pathways of N input into ecosystems. We tried to use this approach to assess the main input pathways of nitrogen into the sand dune area of the north-western Negev Desert (Israel). The following two pathways are the main sources for nitrogen input into the system:

- i. Biological fixation of atmospheric nitrogen by cyanobacteria present in biological crusts and by N_2 -fixing vascular plants (e.g. the shrub *Retama raetam*);
- ii. Atmospheric input of nitrogen by wet deposition with rainfall, dry deposition of dust containing N compounds, and gaseous deposition.

Samples were taken from selected environmental compartments such as biological crusts, sand underneath these crusts (down to a depth of 90 cm), N_2 -fixing and non- N_2 -fixing plants, atmospheric bulk deposition as well as soil from arable land north of the sandy area in three field campaigns in March 1998, 1999 and 2000. The $\delta^{15}\text{N}$ values measured were in the following ranges: grass -2.5‰ to $+1.5\text{‰}$; *R. raetam*: $+0.5\text{‰}$ to $+4.5\text{‰}$; non- N_2 -fixing shrubs $+1\text{‰}$ to $+7\text{‰}$; sand beneath the biological crusts $+4\text{‰}$ to $+20\text{‰}$ (soil depth 2–90 cm); and arable land to the north up to 10‰ . Thus, the natural ^{15}N abundance of the different N pools varies significantly. Accordingly, it should be feasible to assess different input pathways from the various ^{15}N abundances of nitrogen. For example, the biological N fixation rates of the Fabaceae shrub *R. raetam* from the ^{15}N abundances measured were calculated to be 46–86% of biomass N derived from the atmosphere. The biological crusts themselves generally show slight negative ^{15}N values (-3‰ to -0.5‰), which can be explained by biological N fixation. However, areas with a high share of lichens, which are unable to fix atmospheric nitrogen, show very negative values down to -10‰ . The atmospheric N bulk deposition, which amounts to 1.9–3.8 kg N/ha yr, has a ^{15}N abundance between 4.4‰ and 11.6‰ and is likely to be caused by dust from the arable land to the north. Thus, it cannot be responsible for the very negative values of lichens measured either. There must be an additional N input from the atmosphere with negative $\delta^{15}\text{N}$ values, e.g. gaseous N forms (NO_x , NH_3). To explain these conflicting findings, detailed information is still needed on the wet, particulate and gaseous atmospheric deposition of nitrogen.

Keywords: Desert sand dune; Ecosystems; Nitrogen 15; Nitrogen input

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INTRODUCTION

The total biotic and abiotic N pool size of desert ecosystems is lower than in most other ecosystems [1]. Apart from water, nitrogen also controls primary production and thus all the other biological activities in desert ecosystems [2, 3]. In the case of precipitation greater than 100 mm/yr, nitrogen appears to be the actual limiting factor for biological activity and plant primary production in the Israeli Negev Desert [4, 5].

Two main pathways can be considered for N input into this system [1, 2, 6–8]:

- i. Biological fixation of atmospheric nitrogen (BNF) by:
 - cyanobacteria present in biological crusts [6, 8, 9–12];
 - legume–*Rhizobium* symbioses, *e.g.* the Fabaceae shrub *Retama raetam* [13, 14];
 - associative symbiotic N fixation by free-living microorganisms [1, 2, 8].
- ii. Atmospheric deposition of nitrogen [1, 15–16] as:
 - wet deposition in rainfall;
 - dry deposition of dust containing N compounds, possibly from arable land adjacent to the north;
 - gaseous deposition by the direct uptake of NO_x and NH_3 .

The variation of the natural ^{15}N abundance is often used to examine the pathways of N input and transformation into an ecosystem ([17–25], current reviews [26, 27]). The great advantage of the natural ^{15}N abundance method is that it can be easily applied anywhere without the need for additional ^{15}N labelling, which could disturb the system investigated. The disadvantages of this method are caused by the relatively low natural variation of ^{15}N in the soil/plant system of at most 50‰ [27] combined with isotopic effects (isotopic discrimination), which may place limitations on this approach [26–29] as briefly elucidated as follows. The ^{15}N abundance of the pool under consideration depends on:

- i. the ^{15}N abundance of the N source supplying the pool of interest;
- ii. isotopic effects of the subsequent N transformation in the target pool combined with N losses from the target pool.

The effects of ii. may alter the ^{15}N abundance deriving from the N source. For example, although N mineralisation in soil leads to ^{15}N depletion of the ammonium formed, subsequent nitrification – which has a greater isotopic effect than mineralisation – leads to the enrichment of the remaining ammonium. Hence, depending on the conditions in the soil, the $\delta^{15}\text{N}$ value of ammonium may exceed that of the feeding organic matter pool. As a result, the conclusion drawn from the ^{15}N abundance measured alone may be somewhat uncertain or ambiguous. But, the opportunity to use ^{13}C additionally to ^{15}N to improve the results was not given in this investigation.

In the following, we present the variation of the natural ^{15}N abundance of selected N pools from four different locations across the sand dune field of the north-western Negev Desert in Israel and some evaluations regarding different N input pathways.

MATERIALS AND METHODS

Study Area

The study area is located on the southern margin of the easternmost extension of the large continental Sinai-Negev sand sheet, about 45 km inland from the Mediterranean Sea (Fig. 1).

It consists of four sampling locations (SL 1–4) across a transect from north (SL 1) to south (SL 4). The area borders to the north on the agricultural area of Gevulot (GE), to the south on the Nizzana Compound, to the east on the Egyptian frontier, and to the west on the Negev Highlands. The 10–15 m high linear dunes mainly show W–E orientation and have undergone several remobilisation phases, the latter largely being due to human impact. This was especially the case between 1967 and 1982 when intensive grazing led to the partial destruction of the vegetation cover and to the reactivation of the dunes, which also changed their morphology [30]. The dune crests are mobile but exhibit relatively low sand transport rates owing to the low winds [16], whereas the dune flanks are relatively stable. In contrast to the Egyptian part, on the Israeli side Bedouin land use has been restricted, since 1982. This has resulted in an increase of shrubs by 180%. Consequently, the dunes here are stabilised with the exception of the mobile dune crests. The vegetation cover varies from 20% on south-facing slopes to 50% on north-facing slopes [31]. Along the slopes, large areas are covered by various cryptogamic crusts that enhance surface stability and greatly control ecosystem processes [16, 31–33].

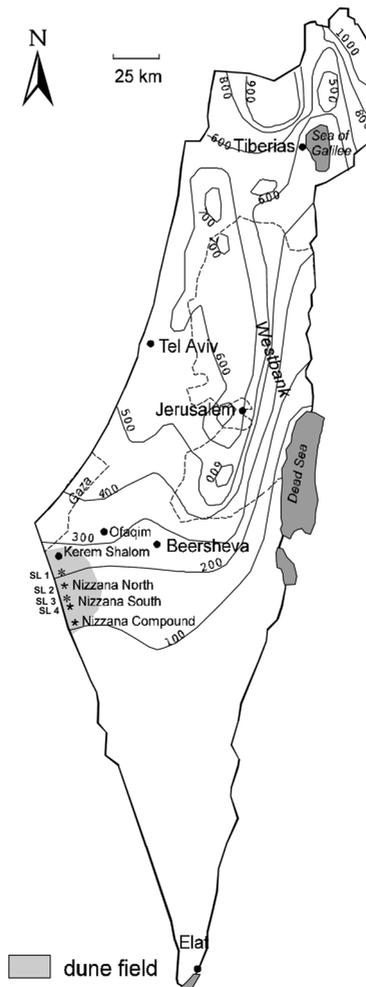


FIGURE 1 Study area in the north-western Negev Desert (Nizzana) with marked SL 1–4.

The local climate is largely determined by a sharp gradient from Mediterranean to hyper-arid Saharo-Arabian climates and by seasonally shifting pressure cells. The mean annual temperature at the Nizzana experimental site is around 20 °C (mean minimum: 12.5 °C; mean maximum: 26.5 °C). Mean rainfall is 90–100 mm, but exhibits high interannual variability [16, 30].

Sampling

Samples from different N pools were taken in March 1998, 1999 and 2000, namely:

- i. The biological crust and the underlying sand down to a depth of 90 cm;
- ii. Lichens: *Fulgensia fulgens*, *Squamarina lentigeria* and *S. crassa*;
- iii. Plants: shrubs: *R. raetam*, *Anabasis articulata*, *Artemisia monosperma* and *Thymelaea hirsuta*; grass: *Bromus fasciculatus*.

The biological crusts were sampled by lifting off the crust with a small spatula and carefully removing sand adhering to the crust's exopolysaccharide filamentous sheaths. The crust samples obtained had a thickness ranging from 1 mm in the southern part (SL 4) to 3–4 mm (SL 2) in the northern part of the dune field. Soil and sand samples down to a depth of 10 cm were taken by a hollow drilling auger after removing the biological crust. All other samples down to a depth of 90 cm were taken from sampling pits (only in 1999). Plant samples were taken by cutting leaves from different parts of the shrubs and grass. Samples of the crust, soil and grass were taken in three replicates from two different plots at each location. Shrub samples were cut from two different tacked shrubs at each location. In 2000, samples were taken only from plants at a few plots to confirm the measurements in 1998 and 1999.

The atmospheric N deposition was measured using bulk collectors [16]. The collected samples were acidified with sulphuric acid for storage and transportation to Germany.

Sample Preparation and ¹⁵N Analysis

The plant material was coarsely cut and dried at 65 °C. The dry matter was ground to a fine powder with a rotary mill (Retsch ZM 1, Germany).

The soil or sand samples were dried at 105 °C and then homogeneously milled with a ball mill (Retsch MM 2, Germany).

The ¹⁵N in the pre-treated samples was measured on an isotope mass spectrometer DELTA S (Finnigan MAT Bremen, Germany) coupled online with an elemental analyser CHN EA 1108 (Carlo Erba, Italy).

The bulk samples were treated by Kjeldahl digestion followed by steam distillation to isolate the ammonium N. The ¹⁵N of the isolated ammonium was again measured on an elemental analyser–IRMS coupling (see above) [34, 35].

The natural ¹⁵N abundances measured are usually given in δ‰ units.

$$\delta\text{‰} = \frac{R_s - R_o}{R_o} \times 1000 \quad (1)$$

R_s – ¹⁵N/¹⁴N ratio of sample;

R_o – ¹⁵N/¹⁴N ratio of atmospheric N₂.

Calculation of the Biological N Fixation of *Retama raetam*

A widespread approach to determine the BNF of legume–*Rhizobium* symbioses is the natural ^{15}N abundance method [20, 21, 26, 27, 36, 37]. Because the $\delta^{15}\text{N}$ signature of nitrogen derived from soil is commonly distinct from nitrogen derived from the atmosphere (NdfA), the NdfA can then be calculated using a two-pool model from the quotient of the natural ^{15}N abundances of the N_2 -fixing plant and a reference of the soil N pool using the following equation:

$$\text{NdfA} = \frac{\delta_r - \delta_f}{\delta_r - \delta_b} \times 100 \quad (2)$$

δ_f – $\delta^{15}\text{N}$ of N_2 -fixing plant in ‰

δ_r – $\delta^{15}\text{N}$ of reference sample in ‰

δ_b – isotopic shift against air's $\delta^{15}\text{N}$ signature by the BNF itself.

The calculation is explained in more detail in Shearer and Kohl [20], and a current review for woody plants is given in Boddey *et al.* [37].

The precision of this approach primarily depends on the difference between the ^{15}N in the soil and the atmospheric pool. With a typical soil N enrichment of 8‰ and a precision of ^{15}N measurement of 0.2‰ (1σ), the detection limit is 9‰ NdfA. Because representatively determining the plant available soil N is difficult, a non- N_2 -fixing reference plant growing at the same site with a similar root system and temporal N uptake pattern to the N_2 -fixing plant is often used. However, frequently these assumptions are not met. Hence, the ^{15}N abundance of the reference plant may differ from the available soil N up to $\pm 3\%$ [38]. Additionally, there is a relatively high detection limit as mentioned above. Therefore, estimates based on the natural ^{15}N abundance method are often only semi-quantitative or only provide an indication that a species is fixing N_2 [26, 27]. But for woody plants in a natural environment this method is the only practical way to assess whether N_2 fixation occurs [21, 37].

RESULTS AND DISCUSSION

The ^{15}N Abundance of Surface N Pools

Figure 2 shows an overview of the ^{15}N abundances of the non- N_2 -fixing plants, crusts and sand close beneath the crust (down to a depth of 5 cm) for the different sampling locations. The columns show the average of two sampling points per location in March 1998 and 1999, respectively. In addition to the sites in the dune field, we determined ^{15}N values from arable soil near GE on the north-eastern margin of the sand dune field. As can be seen, the various pools have very different $\delta^{15}\text{N}$ values. Both biogenic crusts and the annual grass *B. fasciculatus* show negative abundances, *i.e.* a depletion of ^{15}N compared to the natural atmospheric abundance. However, their values become less negative or even positive from north to south, which tallies with the regional rainfall gradient.

As shown later, the deeper layers of sand show relatively high positive values in comparison to the upper soil layer and, consequently, non- N_2 -fixing shrubs that – unlike grass – obtain nitrogen from root uptake in deeper soil layers should have similarly positive values. This is true in the case of *A. articulata*. However, the deep-rooted shrub *A. monosperma* [14] has a strikingly lower positive ^{15}N abundance. This indicates that both species take up their N from different soil depths or different pools. Regarding the latter possibility, there is only one mention in the literature of phyllospheric N fixation from the atmosphere [13].

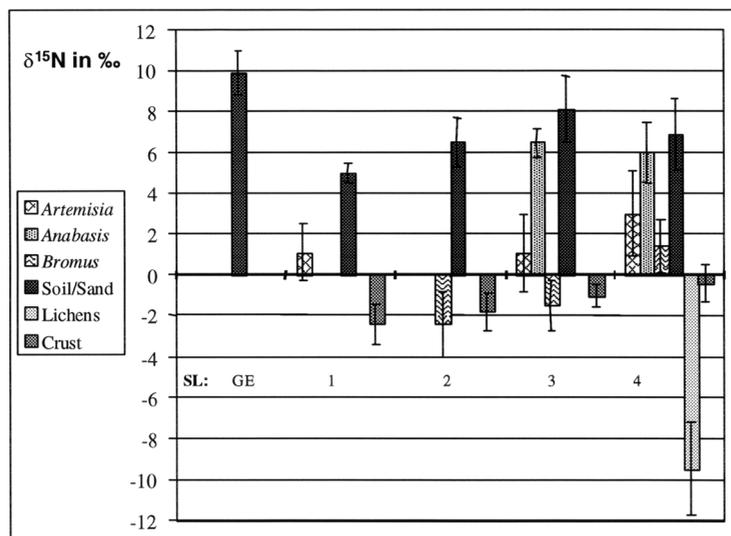


FIGURE 2 Natural ^{15}N abundance in $\delta^{15}\text{N}$ for non- N_2 -fixing plants, biological crusts and sand (0.5–5 cm) at different SL – $\delta^{15}\text{N}$ values are the averages of 1998 and 1999 ($n=6/\text{yr}$, error bars mean single standard deviation 1σ).

Comparing the ^{15}N abundance of the surface crusts separately with the sand below (0.5–5 cm), there is a marked hiatus from about -2‰ in the crusts to $+4$ – 8‰ in the sand (see later Fig. 4). As one major constituent of the biogenic crusts are photoautotrophic cyanobacteria (blue-green algae), a large share of the nitrogen in the crust can be expected to be derived from the atmosphere by BNF. Biological N_2 fixation leads to a slight ^{15}N discrimination, resulting in depletion by up to -2‰ [21, 39]. Our results correspond to this assumption. On the other hand, negative ^{15}N values of grass and extremely negative values (up to -10‰) of non- N_2 -fixing lichens such as *F. fulgens*, *S. lentigera* and *S. crassa* cannot be explained by BNF. Two different hypotheses appear likely to explain this observation:

- i. Absorption of airborne gaseous nitrogen forms such as $\text{NH}_4^+/\text{NH}_3$ and $\text{NO}_3^-/\text{NO}_x$. For this gaseous deposition, negative ^{15}N abundances of -3‰ to -5‰ ($\text{NH}_4^+/\text{NH}_3$, cf. [40]) and of -3‰ to -13‰ ($\text{NO}_3^-/\text{NO}_x$, cf. [41, 42]) have been reported. Unfortunately there are no measurements of $\delta^{15}\text{N}$ values of gaseous deposition for the study area. We measured only the amount and ^{15}N abundance of the bulk deposition samples collected. However, the bulk deposition of N compounds chiefly occurs as singular events with very high variance. The bulk deposition measured is very low and mainly shows positive $\delta^{15}\text{N}$ values (Fig. 3). Only in the southern part of the dune field (SL 4) some slightly negative values were observed. The positive $\delta^{15}\text{N}$ values are probably caused by dust from the arable land in the north which have $\delta^{15}\text{N}$ values of up to $+10\text{‰}$. However, the results may have been strongly falsified by bird droppings, which can have very high $\delta^{15}\text{N}$ values [43] (even though visibly contaminated samples were discarded).
- ii. The increase in $\delta^{15}\text{N}$ values of the biogenic crusts from north to south coincides with a decrease in crustal thickness and with the non-linear decrease in rainfall [44]. The N compounds contained in the rainwater also commonly show negative ^{15}N abundance [41, 42], complying with the hypothesis that highly negative $\delta^{15}\text{N}$ values in the lichens of the surface may also result from rainwater infiltration. The thicker the biogenic crust, the better the retention of rainwater within the crust [32, 44], the higher the N content of the crust, and the more negative the $\delta^{15}\text{N}$ of this nitrogen.

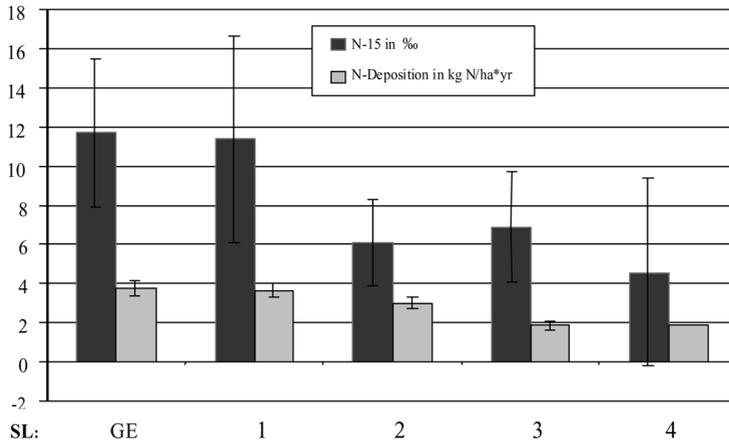


FIGURE 3 Average of amount and ^{15}N abundance for N bulk deposition at different locations for the time span July 1998–June 1998 (sampling frequency 14 d).

N Content and ^{15}N Signature of Soil Depending on the Depth

The main soil type covering about 80% of the dune study area are arenosols, especially calcareous arenosols, a sandy soil with less than 8% clay and more than 2% calcium carbonate. The nitrogen content of this sandy soil is very low. Typical values of the upper soil layer are between 0.013% and 0.0016% on a dry weight basis [45]. But surrounding shrubs nutrient-enriched patches are sometimes observed with much higher N levels in the upper soil layer [7] (“fertile islands” according to Garner and Steinberger, [46]). Figure 4 shows the investigated N content and ^{15}N abundances of the soil at various soil depths and different locations as well. It is known that under regular circumstances the N content decreases with soil depth. By contrast, the ^{15}N abundance increases with soil depth by 5–10‰ [1, 47, 48]. This relationship was also found in our investigations of desert soil.

The main input pathway of nitrogen into the soil is via the biological crust, *i.e.* the nitrogen is slightly ^{15}N depleted due to BNF by cyanobacteria. Nevertheless, as shown in Figure 4, increasingly positive $\delta^{15}\text{N}$ values occur with increasing soil depth. This finding can be explained only by isotopic discrimination during N transformation processes in the soil combined with N losses. The majority of nitrogen in sandy soil is in organic form. Mineralisation converts it into plant-available inorganic nitrogen. As pointed out above (Introduction), mineralisation entails a clear isotopic effect [26, 27, 47, 48], *i.e.* the remaining organic nitrogen becomes more positive. Gaseous N losses occurring from the mineralized inorganic nitrogen – which is depleted against organic nitrogen – lead to further increasing ^{15}N abundance in the total nitrogen of the soil. This means that the more organic N is mineralized and transported out of the system by gaseous losses, the lower the remaining N content in the soil and the more positive the ^{15}N abundance.

Biological N Fixation by the Fabaceae Shrub *Retama raetam*

Biological N fixation by the legume–*Rhizobium* symbioses of the shrub *R. raetam* is the second main N input pathway. Figure 5 shows the $\delta^{15}\text{N}$ values of the N_2 -fixing shrub *R. raetam* and the non- N_2 -fixing reference plant *A. articulata* or the soil itself. *Retama* shows significantly less ^{15}N enrichment compared to the non- N_2 -fixing reference plant *A. articulata* or the soil, proving the uptake of atmospheric nitrogen by BNF.

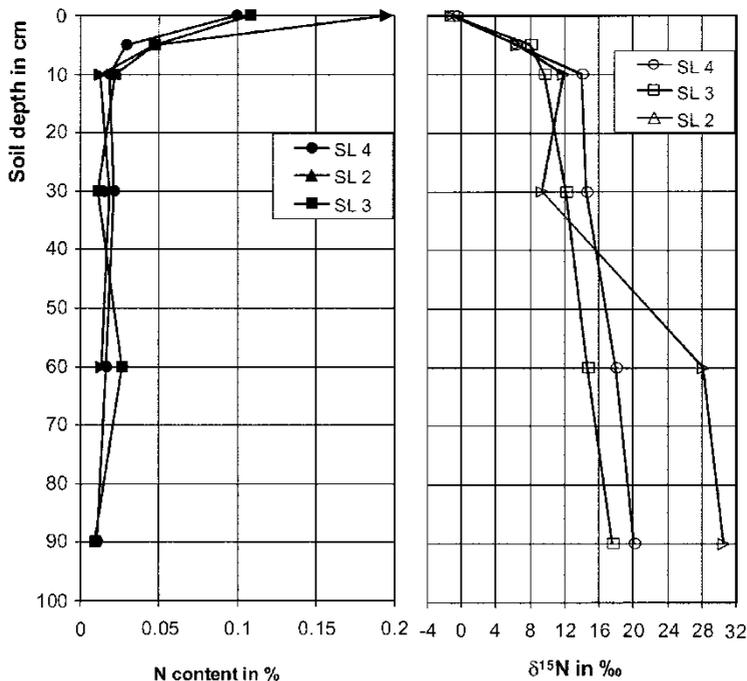


FIGURE 4 N content and ^{15}N abundance depending on the soil depth for one sampling in 1999.

Although we could use only one non- N_2 -fixing plant or had to use the soil itself as reference, we calculated NdfA values, *i.e.* the relative contribution of BNF to the biomass production of *R. raetam* according to Eq. 2 (Tab. I). This means, despite a moderate statistical error for environmental systems ($\text{RSD} \leq 26\%$), the values calculated are relatively uncertain. Additionally, the isotopic ^{15}N shifting by BNF for *R. raetam* is not known and could therefore not be used in the BNF calculations. Taking into account a slightly negative figure as often reported for the isotopic ^{15}N shifting by BNF [37], the results in Table I ought to be

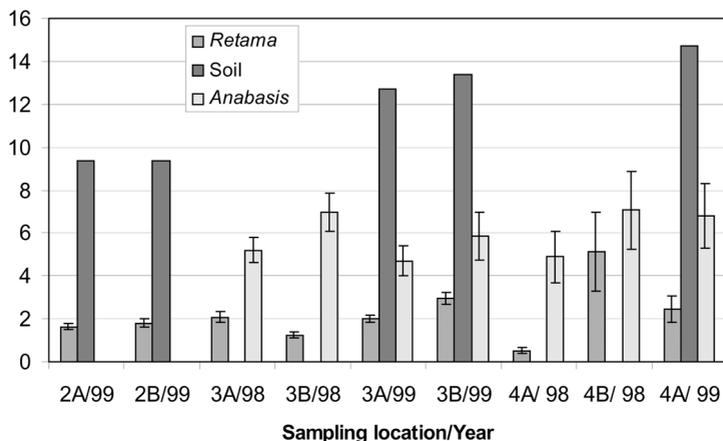


FIGURE 5 ^{15}N -abundance of BNF relevant N pools at different sampling locations (error bars for plants mean single standard deviation 1σ , $n=6$).

TABLE I Biological N Fixation of *R. raetam* as Relative Ndfa for Different Locations and Years.

<i>Location</i>	<i>Year</i>	<i>References</i>	<i>Mean Ndfa ± SD in %</i>	<i>Replicates n</i>
2	1999	S	81.6 ± 1.1	4
3 A	1998	A	83.5	1
3 B	1998	A	85.9 ± 4.7	3
3 A	1999	S, A	71 ± 13	5
3 B	1999	S, A	64 ± 13	5
4 A	1998	A	89 ± 16	3
4 B	1998	A	46 ± 12	3
4 A	1999	S, A	73.5 ± 9.4	5

Note: S, soil; A, *A. articulata*, SD, single standard deviation 1σ .

somewhat lower. Nevertheless, the Ndfa values calculated are surprisingly high, but exhibiting great variation depending on location and time.

The figures in Table I are relative values. They do not say anything about the absolute N input into the ecosystem. To calculate this, one needs to know the production of biomass per area and year for *R. raetam* and the mean N content of it, as well. As these values are not yet available, this calculation must be left to a later publication.

CONCLUSION

- i. The various N pools (fixing and non-fixing plants, biological crusts as well as soil/sand) show significantly different natural ^{15}N abundances. Therefore, the method of natural ^{15}N abundances can be used to evaluate the main input pathways of nitrogen into the sand dune area of the north-western Negev Desert (Israel).
- ii. It was shown that photoautotrophic cyanobacteria (blue-green algae) within the biological crusts fix atmospheric nitrogen. However, quantification of crustal BNF under field conditions is not yet feasible as the biogenic crusts, especially soil lichens, additionally absorb strongly negative airborne nitrogen in either gaseous form or by rainwater uptake.
- iii. *Retama raetam* shows fixation rates of atmospheric nitrogen between 46% and 86%, depending on location and year.
- iv. The bulk deposition is very low (1.9–3.8 kg N/ha × yr) and has mostly positive $\delta^{15}\text{N}$ values. It is probably caused by dust from the arable land located to the north of the study area.
- v. The ^{15}N abundances of the biological crust and the bulk deposition are somewhat conflicting. Detailed information on wet, particulate and gaseous atmospheric deposition of nitrogen is still needed for a further assessment of crustal fixation rates.

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