Biology and Fertility of Soils
https://doi.org/10.1007/s00374-024-01827-8

ORIGINAL PAPER

Root mucilage nitrogen for rhizosphere microorganisms under drought

Meisam Nazari[1] · Samuel Bickel[2] · Yakov Kuzyakov[3] · Nataliya Bilyera[4] · Mohsen Zarebanadkouki[5] · Birgit Wassermann[2] · Michaela A. Dippold[4]

Received: 20 January 2024 / Revised: 24 April 2024 / Accepted: 26 April 2024

Abstract

Nitrogen (N) is a crucial nutrient for the growth and activity of rhizosphere microorganisms, particularly during drought conditions. Plant root-secreted mucilage contains N that could potentially nourish rhizosphere microbial communities. However, there remains a significant gap in understanding mucilage N content, its source, and its utilization by microorganisms under drought stress. In this study, we investigated the impact of four maize varieties (DH02 and DH04 from Kenya, and Kentos and Keops from Germany) on the secretion rates of mucilage from aerial roots and explored the origin of mucilage N supporting microbial life in the rhizosphere. We found that DH02 exhibited a 96% higher mucilage secretion rate compared to Kentos, while Keops showed 114% and 89% higher secretion rates compared to Kentos and DH04, respectively. On average, the four maize varieties released 4 µg N per root tip per day, representing 2% of total mucilage secretion. Notably, the natural abundance of ^{15}N isotopes increased (higher $\delta^{15}N$ signature) with mucilage N release. This indicates a potential dilution of the isotopic signal from biological fixation of atmospheric N by mucilage-inhabiting bacteria as mucilage secretion rates increase. We proposed a model linking mucilage secretion to a mixture of isotopic signatures and estimated that biological N fixation may contribute to 45 - 75% of mucilage N per root tip. The N content of mucilage from a single maize root tip can support a bacterial population ranging from 10^7 to 10^{10} cells per day. In conclusion, mucilage serves as a significant N-rich resource for microbial communities in the rhizosphere during drought conditions.

Keywords Isotopic approaches · Rhizosphere processes · Root exudates · Nitrogen fixation · *Zea mays* L

Introduction

Nitrogen (N) is an essential component of microbial cells including cell walls, proteins, nucleic acids, and enzymes (Nelson et al. 2016; Séneca et al. 2021). In soil, N is essential to maintain crop yields and is often a limiting nutrient that controls the growth and activity of soil microorganisms. In addition to the application of mineral fertilizers, N-increasing strategies based on plant selection and beneficial microorganisms are employed. However, low soil water content due to drought reduces N utilization by microbial communities (Jansson and Hofmockel 2020). In response to dry conditions, soil microorganisms may activate genes associated with organic matter decomposition and biological N fixation to adapt to limited N availability (Yu et al. 2018). A unique strategy of soil microorganisms under drought can be the acquisition of N from the plant (e.g., rhizodeposits) (Wichern et al. 2007, 2008) and the atmosphere (e.g., fixation). Yet, the proportions of N derived from the plant and the atmosphere remain unknown. Root tips of plants secrete mucilage, a viscoelastic high-molecular-weight substance that contains polysaccharides, minerals, lipids and proteins with several enzymes capable to degrade soil organic matter and accelerate nutrient mobilization (Ma et al. 2010;

✉ Meisam Nazari
meisam.nazari@agro.au.dk; meisam.nazari1991@gmail.com

[1] Department of Agroecology, Aarhus University, Tjele, Denmark

[2] Institute for Environmental Biotechnology, Graz University of Technology, Graz, Austria

[3] Department of Soil Science of Temperate Ecosystems, Department of Agricultural Soil Science, University of Göttingen, Göttingen, Germany

[4] Geo-Biosphere Interactions, Department of Geosciences, University of Tübingen, Tübingen, Germany

[5] Soil Biophysics and Environmental Systems, Technical University of Munich, Freising, Germany

Published online: 08 May 2024

Pozzo et al. 2018; Nazari 2021). Mucilage can also serve as a biofilm matrix, similar to microbial extracellular polymeric substances (EPS), shaping microbial habitats within the rhizosphere (Nazari et al. 2022). Bacteria residing the aerial root mucilage of the Sierra Mixe landrace of maize (*Zea mays* Y.) supply 29 - 82% of the plant's N nutrition through biological N fixation (Van Deynze et al. 2018; Amicucci et al. 2019; Bennett et al. 2020). The high viscosity of mucilage enhances soil liquid-phase connectivity and water content in the rhizosphere during drought conditions (Young 1995; Carminati et al. 2010; Benard et al. 2018). Mucilage has the capacity to retain water up to 25 to 600 times its dry weight, thus enhancing the water-holding capacity of the rhizosphere and mitigating the rapid decline in soil hydraulic conductivity during drying (Huang and Gutterman 1999; Kroener et al. 2014; Nazari et al. 2020).

The unique characteristics and functions of root mucilage may facilitate the provision of N to soil microorganisms during drought. This is due to its composition of N-rich compounds (Mary et al. 1993; Nazari 2024), ability to retain soil moisture (Carminati et al. 2010), and susceptibility to rapid decomposition (Ahmed et al. 2018). However, there is limited information available regarding mucilage N, its source, and the mechanisms by which microorganisms can utilize it for their growth and activity under drought. We conducted an investigation using four maize (*Zea mays* L.) varieties to assess their impact on mucilage secretion and to trace the origin of mucilage N supporting microorganisms in the rhizosphere. These four maize varieties were sourced from arid, semi-arid, and temperate agroecological zones, and are expected to exhibit specific adaptations to drought conditions that influence mucilage secretion from their aerial roots. Maize aerial roots, initially aboveground, swiftly transit to belowground roots within a few days. Consequently, mucilage secreted from aerial roots closely resembles that of belowground roots. Furthermore, distinct plant-selection histories may influence the transmission of N-fixing bacteria (Gao et al. 2023; Wassermann et al. 2023) along with other plant traits such as mucilage secretion. Therefore, we anticipate variations in mucilage quantities, properties, and N content among the four maize varieties.

Given that mucilage enhances the water content of the rhizosphere (Carminati et al. 2010), we hypothesized that the secretion rate and water-holding capacity of maize aerial root mucilage are higher in varieties bred for cultivation in arid and semi-arid regions, as an adaptation to dry environments. Despite mucilage containing N in the form of proteins and free amino acids (Van Gelder et al. 2023), the quantities and implications for rhizosphere microorganisms remain unknown. We hypothesized that mucilage contains a sufficient amount of N to support microbial growth and activity in the rhizosphere. Considering the vital role of biological N fixation by mucilage-associated bacteria in supplying N to the plant (Van Deynze et al. 2018), it is plausible that a substantial portion of mucilage N originates from biological (bacterial) N fixation from the atmosphere. Therefore, we hypothesized that mucilage N is largely a product of biological fixation from the atmosphere.

Materials and methods

Soil and plant preparation

We conducted a pot experiment as a randomized complete block design with six replicates in a controlled plant growth chamber. The experiment involved two maize varieties sourced from Kenya (DH02 and DH04) and two maize varieties from Germany (Kentos and Keops). DH02 and DH04 are varieties developed by the Kenya Seed Company in Kitale, Kenya, specifically bred for cultivation in arid and semi-arid regions of Kenya. Kentos and Keops are high-yielding maize varieties developed by the KWS SAAT Company in Einbeck, Germany, intended for cultivation in humid temperate regions of Europe. The soil used in the experiment was a loamy Luvisol, collected from a depth of 0-25 cm on a farm situated in Hohenpölz, Bavaria, Germany. It consisted of 36% sand, 42% silt, and 22% clay. The soil had an organic carbon (C) content of 1.77%, total N content of 0.19%, and microbial biomass C of 490 μg g^{-1}. It exhibited a water-holding capacity of 63% and a pH of 6.4 (Nazari et al. 2023).

Pots measuring 30 cm in height and 15 cm in diameter were uniformly filled with approximately 4 kg of dry soil sieved to a particle size of 2 mm. Seeds of the maize varieties were pre-germinated on wet filter paper for three days before planting, with one seedling placed at a depth of 3 cm in each pot. The plants were cultivated in a growth chamber with a 12-hour photoperiod alternating between day and night. The average temperature and relative humidity in the growth chamber were maintained at 26 ± 1 °C and 60 ± 5%, respectively. Illumination was provided by 243W light-emitting diodes (LEDs) designed to mimic near-daylight spectral composition (Kind LED Growth Lights, California, USA). The soil water content was maintained at 70% of the water-holding capacity for approximately two weeks from sowing until the onset of stem elongation (BBCH30) (Meier 2018). Subsequently, drought conditions were induced by reducing the soil water content to 30% of the water-holding capacity for one week, spanning from the beginning of stem elongation (BBCH 30) to the growth stage nine or more nodes visible (BBCH 39).

Mucilage sampling

Mucilage samples were collected from the aboveground aerial roots of the maize varieties at the growth stage nine

or more nodes visible (BBCH 39), following the method outlined by Ahmed et al. (2015). Initially, the emerged aerial roots were submerged in distilled water for 24 hours to ensure maximum mucilage hydration. Subsequently, the hydrated mucilage (Fig. 1) was carefully extracted from the aerial root tips using a 5 ml pipette. Forceps were employed to collect any remaining mucilage from the aerial roots. The collected mucilage samples were then transferred to 50 ml vials, weighed, and freeze-dried using a freeze-dryer (Beta 1-8 LSCplus, Christ, Osterode, Germany). The mucilage secretion rate was quantified as the dry weight of freeze-dried mucilage per aerial root tip per day. Additionally, the mucilage water-holding capacity was determined by calculating the weight difference between fully hydrated mucilage and freeze-dried mucilage, divided by the weight of freeze-dried mucilage, and expressed as a multiple of its dry weight.

Measurement of mucilage N release and ^{15}N natural abundance

We conducted an analysis of N release and ^{15}N natural abundance (expressed as δ^{15}N, ‰) of freeze-dried mucilage. Freeze-dried mucilage samples (~ 0.5 mg) were carefully weighed into tin capsules measuring 9 mm × 12 mm. The N release within the mucilage was determined using a Flash EA 1112 organic elemental analyzer (Thermo Electron, Milan, Italy). Subsequently, the δ^{15}N of the mucilage N was measured using a Delta XP isotope ratio mass spectrometer (Thermo Electron, Bremen, Germany). The δ^{15}N analysis was conducted at the Centre for Stable Isotope Research and Analysis of the Georg-August University of Göttingen. We used the following assumptions to distinguish between atmospheric and plant-derived N: 1) plants that acquire N from the atmosphere through biological N fixation exhibit low and negative δ^{15}N values (slightly depleted in ^{15}N) and 2) plants that directly uptake N from the soil have high and positive δ^{15}N values (enriched in ^{15}N) (Boddey et al. 2001; Van Deynze et al. 2018).

Fig. 1 Aerial root mucilage from the DH02 maize variety. The image depicts the hydrated mucilage following immersion of the aerial root in distilled water for 24 hours

Estimation of biological N fixation in mucilage

We employed an additive mixing model to establish the relationship between the mucilage N release rate of a root tip (Q_M) and the mucilage N isotopic signature ($\delta^{15}N_M$). This model involved partitioning the flux between the plant-exuded N ($\delta^{15}N_P$) with high (positive) values and the atmospherically fixed N ($\delta^{15}N_F$) with low (negative) values. Under this model, we assumed that the N flux consisted of the plant-exuded N (Q_P) and the fixed N (Q_F), which remained constant throughout the experiment ($Q_M = Q_P + Q_F$). Given a steady-state flux, the N isotopic composition in mucilage is described by equation (1):

$$\delta^{15}N_M = \frac{\delta^{15}N_P\left(Q_M - Q_F\right) + \delta^{15}N_F Q_F}{Q_M} \quad (1)$$

The equation was fitted using $\delta^{15}N_M$ and Q_M data to estimate Q_F, $\delta^{15}N_P$, and $\delta^{15}N_F$. The fit was constrained to ensure Q_F positive. We assessed the uncertainty of the model fit and the estimated quantities through bootstrapping ($n = 1000$).

Statistical analysis

Data analysis was conducted using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA). Normality and homogeneity of variances were assessed using the Shapiro-Wilk and Levene's tests, respectively. In cases where these assumptions were not met, data were transformed using the square root function. One-way analysis of variance (ANOVA) was employed to assess significant effects among maize varieties at a significance level (α) of 0.05. Pair-wise comparisons of means between treatments were performed using Tukey's HSD (Honestly Significant Difference) test at $\alpha = 0.05$. However, for data on mucilage secretion rate and mucilage N release, which did not meet the assumptions of homogeneity of variances and normality even after transformation, Welch's F-test of unequal variances and the non-parametric Kruskal–Wallis test were utilized, respectively, at $\alpha = 0.05$. Pair-wise comparisons of means for mucilage N release were conducted using the Kruskal–Wallis test. Boxplots were generated using SigmaPlot 14.0 (Systat, San José, CA, United States). All reported results represent arithmetic means of six replicates ($n = 6$).

Results

Mucilage secretion rate and water-holding capacity

On average, the DH02 variety secreted 96% more mucilage than the Kentos variety (Fig. 2a). The Keops variety exhibited a 114% and 89% higher mucilage secretion compared to the Kentos and DH04 varieties, respectively. Regarding water-holding capacity, mucilage produced by the Keops and Kentos varieties demonstrated 148% and 105% lower values, respectively, compared to mucilage from the DH04 variety (Fig. 2b).

Mucilage N release and ^{15}N natural abundance

The mucilage N release of the varieties DH02 and Keops were 30% and 26% higher compared to the mucilage N release of the variety Kentos, respectively (Fig. 3a). The lowest $\delta^{15}N$ (- 1.25 ‰) belonged to the variety Kentos, which differed from the $\delta^{15}N$ of the other varieties ranging from 3.70 ‰ to 3.92 ‰ (Fig. 3b).

Biological N fixation in mucilage

The $\delta^{15}N$ in the mucilage exhibited an increase with mucilage N release per root tip (Fig. 4). This trend suggests that the signal from atmospheric $\delta^{15}N$ is diluted with increasing mucilage secretion, primarily composed of plant-exuded $\delta^{15}N$. The additive mixing model, incorporating

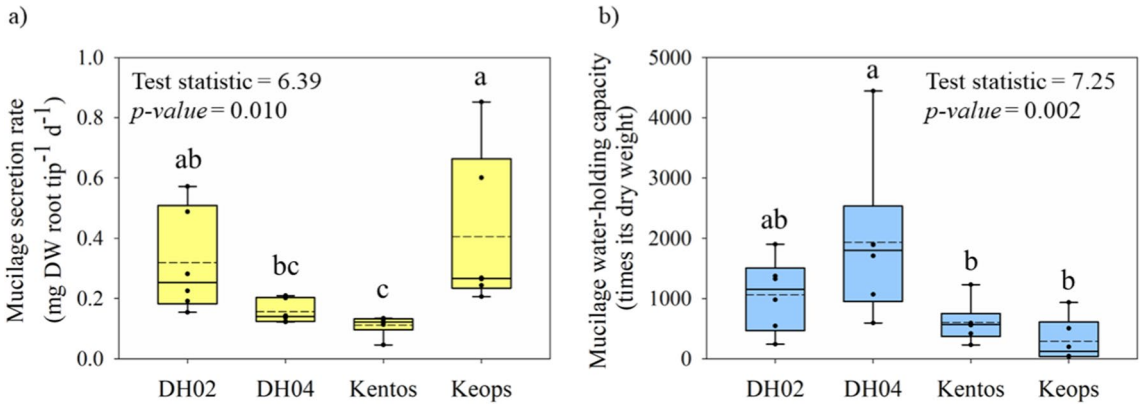

Fig. 2 Mucilage secretion rate (**a**) and mucilage water-holding capacity (**b**) of aerial roots of maize varieties sourced from Kenya (DH02 and DH04) and Germany (Kentos and Keops) at the growth stage nine or more nodes visible (BBCH 39). A *p-value* < 0.05 indicates a statistically significant effect of maize variety. Letters on each bar denote statistically significant differences (Tukey's HSD, at $\alpha = 0.05$, $n = 6$). The box represents the 25th and 75th percentiles. Dashed and solid lines show the arithmetic mean and median, respectively

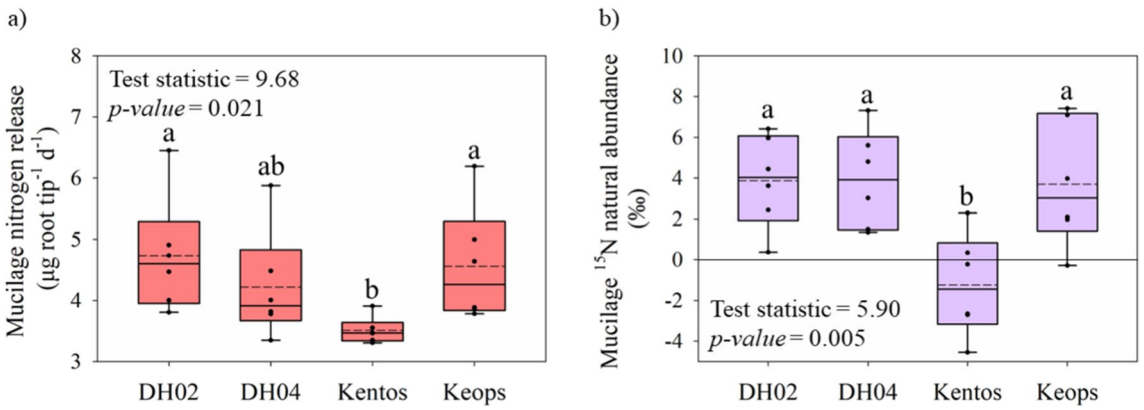

Fig. 3 Mucilage N release (**a**) and mucilage ^{15}N natural abundance ($\delta^{15}N$) (**b**) of maize varieties sourced from Kenya (DH02 and DH04) and Germany (Kentos and Keops) at the growth stage nine or more nodes visible (BBCH 39). A *p-value* < 0.05 indicates a statistically significant effect of maize variety. Letters on each bar denote statistically significant differences (Tukey's HSD for $\delta^{15}N$ and the Kruskal–Wallis pair-wise comparison for mucilage N release, both at $\alpha = 0.05$, $n = 6$). The box represents the 25th and 75th percentiles. Dashed and solid lines show the arithmetic mean and median, respectively

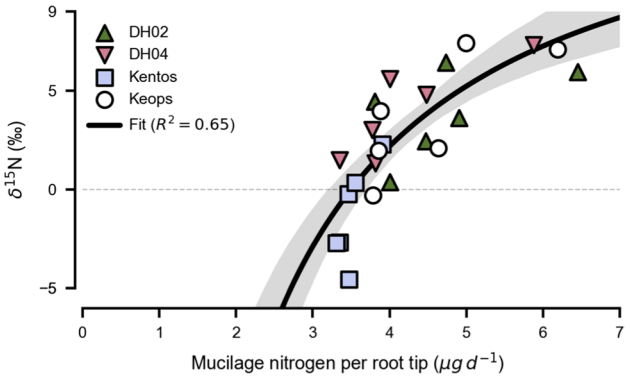

Fig. 4 High mucilage N release rate is associated with high ^{15}N natural abundance (δ^{15}N). An additive mixing model (eq. 1) was fitted to the data from the four maize varieties (n = 24). The shaded area represents the bootstrapped central 95%

three inferred parameters, provided a good fit to the data, explaining a substantial proportion of the variance (R^2 = 0.65). From the model, we derived the following estimates (bootstrapped mean and standard deviation, n = 1000): the flux of biological N fixation per root tip ($Q_F = 3.0 \pm 0.3$ µg d^{-1}) and the isotope signatures of plant-exuded and fixed N ($\delta^{15}N_E = 18 \pm 2$ ‰, and $\delta^{15}N_F = -3 \pm 2$ ‰, respectively).

Discussion

Mucilage secretion rates differ between the maize varieties

The DH02 variety exhibited a higher mucilage secretion rate compared to the Kentos variety (Fig. 2a), consistent with findings from previous field and pot experiments (Nazari et al. 2020, 2023). A drought-tolerant barley variety secreted larger quantities of mucilage from its roots than a drought-susceptible one (Carter et al. 2019). It is plausible that breeding efforts in Kenya, aimed at enhancing drought tolerance, may have favored the increased mucilage secretion rate in DH02, given the putative role of mucilage in drought mitigation. For instance, mucilage sugars such as galactose, fucose, and glucose can serve as energy sources for rhizosphere microorganisms to produce extracellular polymeric substances (EPS) and confer protection against drought (Nazari et al. 2022). Moreover, mucilage and other root exudates may stimulate the proliferation of plant-growth-promoting bacteria, such as *Pseudomonas putida*, thereby enhancing plant survival in water-deficient conditions (Zulfikar Ali et al. 2011).

The increased membrane permeability of root tip cells in maize under drought conditions, facilitating water uptake, has been documented (Ionenko et al. 2010). Hence, another potential factor contributing to the higher mucilage secretion rate in DH02 could be the selection for increased membrane permeability of root tip cells, a trait favored by breeding under drought conditions. However, contrary to our initial expectation, the Keops variety from Germany exhibited a higher mucilage secretion rate than DH04, yet a comparable rate to DH02 (Fig. 2a). This observation suggests that factors beyond drought adaptation may also influence mucilage secretion. We speculate that the breeding of Keops in soils characterized by high microbial activity might have led to its elevated mucilage secretion rate. Similarly, previous research has demonstrated that maize varieties cultivated in a soil with high microbial activity exhibited higher mucilage secretion rates compared to the same varieties grown in a soil with lower microbial activity (Nazari et al. 2023).

Maize varieties from dry regions exhibit higher mucilage water-holding capacity

The mucilage secreted by the Keops and Kentos varieties exhibited a lower water-holding capacity compared to that of the DH04 and DH02 varieties (Fig. 2b). Mucilage water-holding capacity largely depends on uronic acid-calcium ion interactions (Brax et al. 2019), suggesting that the reduced capacity in Keops and Kentos mucilage could be attributed to lower uronic acid or calcium content. The higher mucilage water-holding capacity observed in DH04 and DH02 compared to Keops and Kentos underscores its functional significance in maize drought tolerance. This increased capacity represents a plant strategy aimed at augmenting rhizosphere water content and hydraulic conductivity under water-limited conditions (Carminati et al. 2010; Kroener et al. 2014; Benard et al. 2019). Such enhancement is crucial for maintaining root-soil contact and supporting plant survival by protecting against the destruction of root tissues and root hairs, as well as preventing root shrinkage.

Considering mucilage function as a biofilm matrix (Nazari et al. 2022), a well-hydrated mucilage layer covers more soil particles, providing favorable moist conditions in the rhizosphere for both plants and microorganisms, particularly in dry soils. The notably high water-holding capacity of mucilage suggests its resemblance to superabsorbent polymers, as they both retain similar quantities of water (Ai et al. 2021). This high water-holding capacity of mucilage can facilitate biological N fixation by reducing gas diffusion, thereby creating an anaerobic microenvironment conducive to oxygen-sensitive nitrogenase activity (Nazari 2024). However, it is worth noting that a lower water-holding capacity may prove advantageous in humid soils and under anoxic conditions to prevent restrictions in gaseous transport.

Mucilage is a desirable N source for rhizosphere microorganisms

The four maize varieties secreted an average of 4 µg N per root tip daily (Fig. 3a), which corresponds to nearly 2% of the total mucilage secretion. Additionally, mucilage from all maize varieties exhibited a high water-holding capacity (Fig. 2b). This high water-holding capacity plays a crucial role in maintaining soil moisture in the rhizosphere under drying conditions (McCully and Boyer 1997; Carminati et al. 2010), facilitating microbial assimilation of mucilage N during drought. It is worth noting that microbial N utilization is strongly influenced by water availability (Jansson and Hofmockel 2020). Moreover, the high water-holding capacity of mucilage serves to protect microorganisms from desiccation (Nazari et al. 2022), which can enhance microbial activity and soil organic matter mineralization in the rhizosphere.

Mucilage N exists in the form of proteins and free amino acids (Van Gelder et al. 2023), indicating that rhizosphere microorganisms can enzymatically convert them into mineral forms accessible for microbial and plant uptake. Furthermore, Zarebanadkouki et al. (2019) demonstrated that mucilage facilitates nutrient diffusion in drying soil. This, in turn, promotes the availability of soil mineral N to the plant. These findings underscore the complicated interdependence within the plant holobiont, encompassing the collective genetic and functional traits of the host and its associated microorganisms (Vandenkoornhuyse et al. 2015). In this context, mucilage serves a dual role: supporting the plant's nutrient acquisition while simultaneously fostering microbial communities that contribute to adaptation to harsh environmental conditions.

Source of mucilage N: biological fixation or plant exudation?

The average range of δ15N values between -1.25 to 3.92 ‰ (Fig. 3b) indicate that the N released with the mucilage is a mixture of plant-exuded and biologically fixed N. Interestingly, negative δ15N values have been observed for non-fixing plants (Boddey et al. 2001; Van Deynze et al. 2018). The maize plants in our study likely acquired N from the fertile soil, which had a high N content, in addition to being supplied with atmospheric N through biological fixation. Given that biological N fixation is energetically costly for plants (Bennett et al. 2020), their investment in employing microorganisms for this purpose suggests a strategy to survive and thrive under stress conditions. For example, in N-poor soils, biological N fixation by bacteria residing in the aerial root mucilage of Sierra Mixe maize landrace supplied 29 - 82% of the plant's N requirement (Van Deynze et al. 2018). Microbial inheritance may also influence this process, as maize seed-associated microbes, including those capable of atmospheric N fixation, have been found to persist from wild ancestor plants to present-day domesticated maize, thus being conserved across maize evolution (Johnston-Monje and Raizada 2011; Gao et al. 2023). Nonetheless, maize plants can also uptake N from the soil through their belowground roots, internally process it, and package it within mucilage before secreting it from their aerial roots. Consequently, plants can redistribute soil N to the rhizosphere through root mucilage for microbial utilization during periods of stress, such as drought or N deficiency. Therefore, our study underscores the importance of considering the redistribution of mucilage N to the soil as an overlooked component of the N cycle.

We acknowledge that our simple model for calculating the contribution of mucilage N by N-fixing bacteria has its limitations. The model assumes a steady secretion of mucilage, which is unlikely considering plants' diurnal physiological responses. However, we anticipate relatively constant mucilage secretion rates throughout the measurement period. Additionally, the simple mixing model assumes only two sources of mucilage N (i.e., from exudation and atmospheric fixation) as the main contributors to the mucilage isotopic signature. This assumption may underestimate the loss and degradation of N-containing compounds through other processes, contributing to the uncertainty of the model fit. Despite these simplifications, our model estimates align with previously published values (e.g., Van Deynze et al. 2018), and the model provides mechanistic insight into the dependency of the isotopic signature on mucilage secretion rates.

Potential utilization of mucilage N by microorganisms in the rhizosphere

Comparing the mucilage N release rate with the N requirements of bacterial cells supports our hypothesis that mucilage contains sufficient N for microorganisms to effectively colonize the amize rhizosphere. A bacterial cell typically utilizes between 0.01 and 0.4 femtograms N per hour, although this can vary depending on factors such as growth rate, cell size, and environmental conditions (Prosser 1990; Kuypers et al. 2018). Assuming that C is not limiting in the rhizosphere and all available N is utilized by bacteria due to the favorable N to C ratio (15.7 mg g^{-1}) of mucilage (Mary et al. 1993), we can estimate that a maize root tip could potentially host up to 10^7 to 10^{10} bacterial cells per day. These estimated bacterial abundances are comparable to values observed in the maize rhizosphere (Zhu et al. 2016). Nonetheless, it is noteworthy that N-fixing bacterial genera and families such as *Burkholderia*, *Herbaspirillum*, and *Azospirillumin*, are enriched in the mucilage surrounding aerial roots of both landrace and modern maize varieties (Van Deynze et al. 2018; Gao et al. 2023).

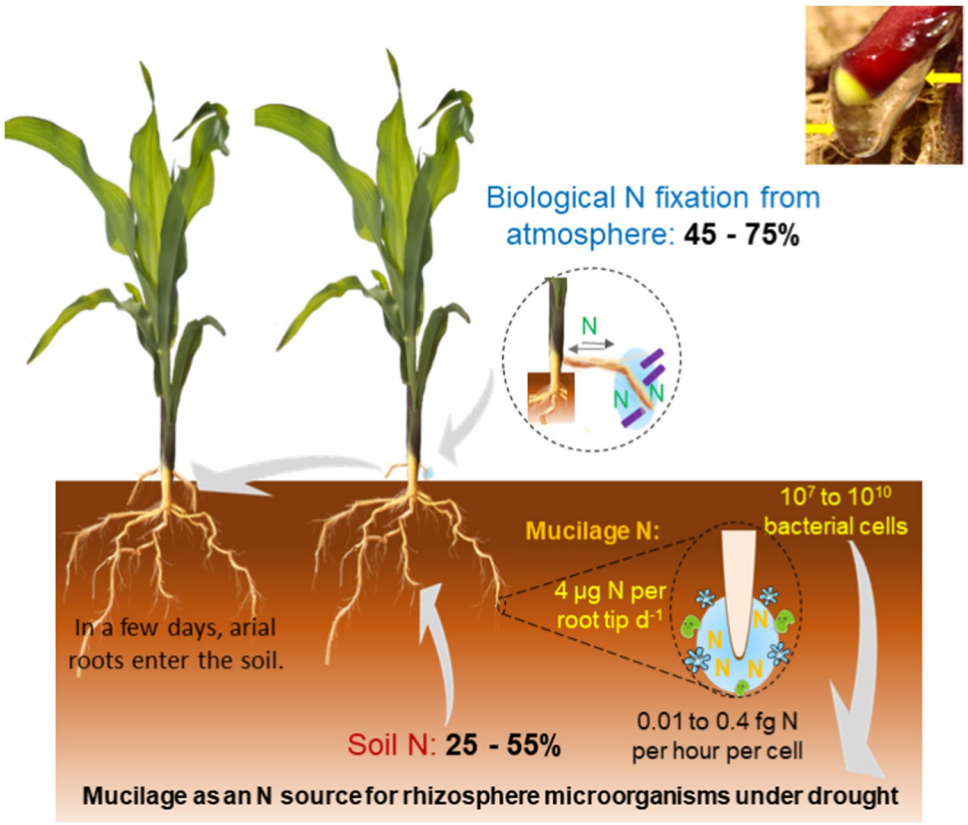

Fig. 5 Schematic representation of N in the aerial root mucilage of maize (*Zea mays* L.), elucidating its origin and its pivotal role in developing an N-rich rhizosphere for bacteria under drought

Our model suggests that up to 75% of mucilage N could originate from biological fixation in the four maize varieties. However, this proportion reduces to about 45% in maize varieties with high mucilage secretion rates. This range is comparable to values reported in earlier studies (e.g., Van Deynze et al. 2018). It is worth noting that as the aerial roots grow deeper into the soil, we anticipate reduced gaseous transport, which may result in lower estimated values.

Our findings have important implications for understanding N dynamics in the rhizosphere and their influence on microbial communities. By demonstrating that maize root mucilage can release substantial amounts of N, with a notable proportion originating from biological N fixation, our research sheds light on a previously underexplored aspect of plant-microbe interactions. The ability of mucilage-derived N to support a bacterial population ranging from 10^7 to 10^{10} cells per day underscores the crucial role of mucilage in sustaining microbial life in nutrient-limited environments, particularly under drought conditions. These findings contribute to a deeper understanding of N cycling in agroecosystems and highlight the potential of plant mucilage as a sustainable strategy for enhancing soil fertility and microbial activity in agricultural systems.

Furthermore, soil microorganisms can completely decompose the secreted mucilage within two weeks (Ahmed et al. 2018). By utilizing mucilage as an energy source, soil microorganisms produce EPS that can protect them and their host against environmental stresses (Nazari et al. 2022). However, the rapid utilization of mucilage compounds by soil microorganisms can lead to a loss of its hydraulic properties (e.g., water-holding capacity) and physical physicl (e.g., viscosity) functions. This necessitates careful consideration of the role of mucilage in regulating plant-soil hydraulics under drought conditions, especially during prolonged dry periods. Specifically, young plant roots need to acquire water (e.g., from rain) and secrete new mucilage, which may not be feasible in rainfed fields experiencing drought.

Conclusions

The variation observed in the secretion rate and water-holding capacity of aerial root mucilage among maize varieties suggests a potential genetic basis for these traits, indicating opportunities for further development through breeding programs. Particularly noteworthy is the high mucilage water-holding capacity exhibited by maize varieties from dry regions, implying a specific role of mucilage in conferring drought tolerance by maintaining moisture in the rhizosphere to the benefit of both plants and microorganisms. Mucilage emerges

as a significant N source for microbial life in the rhizosphere, with a single maize root tip capable of supporting a daily bacterial population ranging from 10^7 to 10^{10} cells. A considerable proportion (45 - 75%) of mucilage N is derived from biological N fixation from the atmosphere (Fig. 5). This underscores the complex interplay between the mucilage microbiota and their plant hosts, suggesting potential avenues for selective breeding of drought-tolerant maize varieties, considering the heritability of traits shaping the rhizosphere microbiome. To advance our understanding and leverage these findings for crop improvement, future research should uncover the specific genetic mechanisms governing mucilage secretion rate and water-holding capacity in maize varieties, especially those adapted to arid regions. Additionally, investigations into the inheritance and transmission of N-fixing bacteria across plant generations will be crucial for developing targeted crop improvement strategies and fostering sustainable agricultural practices.

Acknowledgements We express our gratitude to the German Federal Environmental Foundation (Deutsche Bundesstiftung Umwelt, DBU) for funding MN, as well as the Robert-Bosch Junior Professorship 2017 for supporting MD's research, along with the provision of laboratory consumables for this study. We extend special thanks to Dr. Emmanuel Arthur for his valuable comments on the manuscript. Our appreciation also goes to KWS Germany and ICRISAT Kenya for their contribution of seeds.

Funding Open access funding provided by Aarhus Universitet

Declarations

Competing interests The authors have no conflicts of interest to declare.

References

Ahmed MA, Banfield CC, Sanaullah M, Gunina A, Dippold MA (2018) Utilisation of mucilage C by microbial communities under drought. Biol Fertil Soils 54:83–94. https://doi.org/10.1007/s00374-017-1237-6

Ahmed MA, Holz M, Woche SK, Bachmann J, Carminati A (2015) Effect of soil drying on mucilage exudation and its water repellency: a new method to collect mucilage. J Plant Nutr Soil Sci 178:821–824. https://doi.org/10.1002/jpln.201500177

Ai F, Yin X, Hu R, Ma H, Liu W (2021) Research into the superabsorbent polymers on agricultural water. Agric Water Manag 245:106513. https://doi.org/10.1016/j.agwat.2020.106513

Amicucci MJ, Galermo AG, Guerrero A, Treves G, Nandita E, Kailemia MJ, Higdon SM, Pozzo T, Labavitch J, Bennett AB, Lebrilla CB (2019) Strategy for Structural Elucidation of Polysaccharides: Elucidation of a Maize Mucilage That Harbors Diazotrophic Bacteria. Anal Chem 91:7254–7265. https://doi.org/10.1021/acs.analchem.9b00789

Benard P, Zarebanadkouki M, Brax M, Kaltenbach R, Jerjen I, Marone F, Couradeau E, Velde VJMNL, Kaestner A, Carminati A (2019) Microhydrological Niches in Soils: How Mucilage and EPS Alter the Biophysical Properties of the Rhizosphere and Other Biological Hotspots. Vadose Zo J 18:1–10. https://doi.org/10.2136/vzj2018.12.0211

Benard P, Zarebanadkouki M, Carminati A (2018) Physics and hydraulics of the rhizosphere network. J Plant Nutr Soil Sci 182:5–8. https://doi.org/10.1002/jpln.201800042

Bennett AB, Pankievicz VCS, Ané JMA (2020) A Model for nitrogen fixation in cereal crops. Trends Plant Sci 25:226–235. https://doi.org/10.1016/j.tplants.2019.12.004

Boddey RM, Polidoro JC, Resende AS, Alves BJR, Urquiaga S (2001) Use of the 15N natural abundance technique for the quantification of the contribution of N2 fixation to sugar cane and other grasses. Aust J Plant Physiol 28:889–895. https://doi.org/10.1071/pp01058

Brax M, Schaumann GE, Diehl D (2019) Gel formation mechanism and gel properties controlled by Ca 2+ in chia seed mucilage and model substances. J Plant Nutr Soil Sci 182:92–103. https://doi.org/10.1002/jpln.201800430

Carminati A, Javaux M (2020) Soil rather than xylem vulnerability controls stomatal response to drought. Trends Plant Sci 25:868–880. https://doi.org/10.1016/j.tplants.2020.04.003

Carminati A, Moradi AB, Vetterlein D, Vontobel P, Lehmann E, Weller U, Vogel H, Oswald S (2010) Dynamics of soil water content in the rhizosphere. Plant Soil 332:163–176. https://doi.org/10.1007/s11104-010-0283-8

Carminati A, Vetterlein D (2013) Plasticity of rhizosphere hydraulic properties as a key for efficient utilization of scarce resources. Ann Bot 112:277–290. https://doi.org/10.1093/aob/mcs262

Carter AY, Ottman MJ, Curlango-Rivera G, Huskey DA, D'Agostini BA, Hawes MC (2019) Drought-tolerant barley: II. Root tip characteristics in emerging roots. Agronomy 9:220. https://doi.org/10.3390/agronomy9050220

Gao J, Feng P, Zhang J, Dong C, Wang Z, Chen M, Yu Z, Zhao B, Hou X, Wang H, Wu Z, Jemim RS, Yu H, Sun D, Jing P, Chen J, Song W, Zhang X, Zhou Z, Wu J (2023) Enhancing maize's nitrogen-fixing potential through ZmSBT3, a gene suppressing mucilage secretion. J Integr Plant Biol 65:2645–2659. https://doi.org/10.1111/jipb.13581

Gelder V, Oliveira-Filho K, Messina ER, Venado CD, Wilker RE, Rajasekar J, Ané J, Amthor JS, Hanson AD (2023) Running the numbers on plant synthetic biology solutions to global problems. Plant Sci 335:815. https://doi.org/10.1016/j.plantsci.2023.111815

Greenfield LM, Razavi BS, Bilyera N, Zhang X, Jones DL (2021) Root hairs and protein addition to soil promote leucine aminopeptidase activity of Hordeum vulgare L. Rhizosphere 18:100329. https://doi.org/10.1016/j.rhisph.2021.100329

Huang Z, Gutterman Y (1999) Germination of Artemisia sphaerocephala (Asteraceae), occurring in the sandy desert areas of Northwest China. South Afr J Bot 65:187–196. https://doi.org/10.1016/S0254-6299(15)30972-8

Ionenko IF, Anisimov AV, Dautova NR (2010) Effect of temperature on water transport through aquaporins. Biol Plant 54:488–494. https://doi.org/10.1007/s10535-010-0086-z

Jansson JK, Hofmockel KS (2020) Soil microbiomes and climate change. Nat Rev Microbiol 18:35–46. https://doi.org/10.1038/s41579-019-0265-7

Johnston-Monje D, Raizada MN (2011) Conservation and diversity of seed associated endophytes in Zea across boundaries of evolution, ethnography and ecology. PLoS One 6:e20396. https://doi.org/10.1371/journal.pone.0020396

Kroener E, Zarebanadkouki M, Kaestner A, Carminati A (2014) Non-equilibrium water dynamics in the rhizosphere: How mucilage affects water flow in soils. Water Resour Res 50:6479–6495. https://doi.org/10.1002/2013WR014756

Kuypers MMM, Marchant HK, Kartal B (2018) The microbial nitrogen-cycling network. Nat Rev Microbiol 16:263–276. https://doi.org/10.1038/nrmicro.2018.9

Ma W, Muthreich N, Liao C, Franz-Wachtel M, Schütz W, Zhang F, Hochholdinger F, Li C (2010) The mucilage proteome of maize (Zea mays L.) primary roots. J Proteome Res 9:2968–2976. https://doi.org/10.1021/pr901168v

Mary B, Fresneau C, Morel JL, Mariotti A (1993) C and N cycling during decomposition of root mucilage, roots and glucose in soil. Soil Biol Biochem 25:1005–1014. https://doi.org/10.1016/0038-0717(93)90147-4

McCully ME, Boyer JS (1997) The expansion of maize root-cap mucilage during hydration: 3. Changes in water potential and water content. Physiol Plant 99:169–177. https://doi.org/10.1111/j.1399-3054.1997.tb03445.x

Meier U (2018). Growth stages of mono-and dicotyledonous plants: BBCH Monograph. Open Agrar Repositorium, Quedlinburg https://doi.org/10.5073/20180906-074619

Nazari M (2021) Plant mucilage components and their functions in the rhizosphere. Rhizosphere 18:100344. https://doi.org/10.1016/j.rhisph.2021.100344

Nazari M (2024) The unique rhizosphere of aerial roots. Rhizosphere 29:100867. https://doi.org/10.1016/j.rhisph.2024.100867

Nazari M, Bickel S, Benard P, Mason-Jones K, Carminati A, Dippold MA (2022) Biogels in soils: Plant mucilage as a biofilm matrix that shapes the rhizosphere microbial habitat. Front Plant Sci 12:798992. https://doi.org/10.3389/fpls.2021.798992

Nazari M, Bilyera N, Banfield CC, Mason-Jones K, Zarebanadkouki M, Munene R, Dippold MA (2023) Soil, climate, and variety impact on quantity and quality of maize root mucilage exudation. Plant Soil 482:25–38. https://doi.org/10.1007/s11104-022-05669-x

Nazari M, Riebeling S, Banfield CC, Akale A, Crosta M, Mason-Jones K, Dippold MA, Ahmed MA (2020) Mucilage polysaccharide composition and exudation in maize from contrasting climatic regions. Front Plant Sci 11:587610. https://doi.org/10.3389/fpls.2020.587610

Nelson MB, Martiny AC, Martiny JBH (2016) Global biogeography of microbial nitrogen-cycling traits in soil. PNAS 113:8033–8040. https://doi.org/10.1073/pnas.1601070113

Pozzo T, Higdon SM, Pattathil S, Hahn MG, Bennett AB (2018) Characterization of novel glycosyl hydrolases discovered by cell wall glycan directed monoclonal antibody screening and metagenome analysis of maize aerial root mucilage. PLoS One 13:e0204525. https://doi.org/10.1371/journal.pone.0204525

Prosser JI (1990) Autotrophic Nitrification in Bacteria. Adv Microb Physiol 30:125–181. https://doi.org/10.1016/S0065-2911(08)60112-5

Séneca J, Söllinger A, Herbold CW, Pjevac P, Prommer J, Verbruggen E, Sigurdsson BD, Peñuelas J, Janssens IA, Urich T, Tveit AT, Richter A (2021) Increased microbial expression of organic nitrogen cycling genes in long-term warmed grassland soils. ISME Commun 1:69. https://doi.org/10.1038/s43705-021-00073-5

Van Deynze A, Zamora P, Delaux PM, Heitmann C, Jayaraman D, Rajasekar S, Graham D, Maeda J, Gibson D, Schwartz KD, Berry AM, Bhatnagar S, Jospin G, Darling A, Jeannotte R, Lopez J, Weimer BC, Eisen JA, Shapiro H et al (2018) Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota. PLoS Biol 16:e2006352. https://doi.org/10.1371/journal.pbio.2006352

Vandenkoornhuyse P, Quaiser A, Duhamel M, le Van A, Dufresne A (2015) The importance of the microbiome of the plant holobiont. New Phytol 206:1196–1206. https://doi.org/10.1111/nph.13312

Wassermann B, Cernava T, Goertz S, Zur J, Rietz S, Kögl I, Abbadi A, Berg G (2023) Low nitrogen fertilization enriches nitrogen-fixing bacteria in the Brassica seed microbiome of subsequent generations. J Sustain Agric Environ 2:87–98. https://doi.org/10.1002/sae2.12046

Wichern F, Eberhardt E, Mayer J, Joergensen RG, Müller T (2008) Nitrogen rhizodeposition in agricultural crops: Methods, estimates and future prospects. Soil Biol Biochem 40:30–48. https://doi.org/10.1016/j.soilbio.2007.08.010

Wichern F, Mayer J, Joergensen RG, Müller T (2007) Release of C and N from roots of peas and oats and their availability to soil microorganisms. Soil Biol Biochem 39:2829–2839. https://doi.org/10.1016/j.soilbio.2007.06.006

Young IM (1995) Variation in moisture contents between bulk soil and the rhizosheath of wheat (Triticum aestivum L. cv. Wembley). New Phytol 130:135–139. https://doi.org/10.1111/j.1469-8137.1995.tb01823.x

Yu H, Deng Y, He Z, van Nostrand JD, Wang S, Jin D, Wang A, Wu L, Wang D, Tai X, Zhou J (2018) Elevated CO2 and warming altered grassland microbial communities in soil top-layers. Front Microbiol 9:1790. https://doi.org/10.3389/fmicb.2018.01790

Zarebanadkouki M, Fink T, Benard P, Banfield CC (2019) Mucilage Facilitates Nutrient Diffusion in the Drying Rhizosphere. Vadose Zo J 18:1–13. https://doi.org/10.2136/vzj2019.02.0021

Zhu S, Vivanco JM, Manter DK (2016) Nitrogen fertilizer rate affects root exudation, the rhizosphere microbiome and nitrogen-use-efficiency of maize. Appl Soil Ecol 107:324–333. https://doi.org/10.1016/j.apsoil.2016.07.009

Zulfikar Ali S, Sandhya V, Grover M, Linga VR, Bandi V (2011) Effect of inoculation with a thermotolerant plant growth promoting Pseudomonas putida strain AKMP7 on growth of wheat (Triticum spp.) under heat stress. J Plant Interact 6:239–246. https://doi.org/10.1080/17429145.2010.545147