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Rapid and Sensitive *Nitrosomonas europaea* Biosensor Assay for Quantification of Bioavailable Ammonium *Sensu Strictu* in Soil

NGUYEN MINH DONG,[†] NILS RISGAARD-PETERSEN,[‡,§] JAN SØRENSEN,[†] AND KRISTIAN K. BRANDT*,[†]

Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, DK-1871 Frederiksberg C, Denmark, Department of Marine Ecology, National Environmental Research Institute, University of Aarhus, DK-8600 Silkeborg, Denmark, and Center for Geomicrobiology, Institute of Biological Science, University of Aarhus, DK-8000 Aarhus C, Denmark

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Knowledge on bioavailable ammonium *sensu strictu* (i.e., immediately available for cellular uptake) in soil is required to understand nutrient uptake processes in microorganisms and thus of vital importance for plant production. We here present a novel ammonium biosensor approach based on the lithoautotrophic ammonia-oxidizing bacterium *Nitrosomonas europaea* transformed with a *luxAB* sensor plasmid. Bioluminescence-based ammonium detection was achieved within 10 min with a quantification limit in liquid samples of ~20 μM and a linear response range up to 400 μM. Biosensor and conventional chemical quantification of ammonium in soil solutions agreed well across a range of sample and assay conditions. The biosensor was subsequently applied for a solid phase-contact assay allowing for direct interaction of biosensor cells with soil particle-associated (i.e., exchangeable plus fixed) ammonium. The assay successfully quantified bioavailable ammonium even in unfertilized soil and demonstrated markedly higher ratios of bioavailable ammonium to water- or 2 M KCl-exchangeable ammonium in anoxic soil than in corresponding oxic soil. Particle-associated ammonium contributed by at least 74% and 93% of the total bioavailable pool in oxic and anoxic soil, respectively. The *N. europaea* biosensor should have broad relevance for environmental monitoring of bioavailable ammonium and processes depending on ammonium bioavailability.

Introduction

Knowledge on bioavailable pools of ammonium is required to understand biogeochemical processes and ecological interactions in soils and sediments. Ammonium comprises a major N source for both plants and microorganisms and further constitutes a pollutant of growing environmental importance (*1*). The term bioavailability can be divided into two definitions: "bioavailability *sensu strictu*" and "bioaccessibility". A bioavailable *sensu strictu* compound (hereafter simply termed a 'bioavailable compound') may be defined as "that which is freely available to cross an organism's cellular membrane from the medium the organism inhabits at a given time" (*2*). By contrast, a bioaccessible compound may be defined as "that which is available to cross an organism's cellular membrane from the environment, if the organism has access to the chemical" (*2*). This distinction is important, as only bioavailable compounds are able to directly affect organisms at any given time. Hence, nutrient uptake mechanisms for any growing organism must be able to operate at the prevailing bioavailable concentrations. The definitions given above were meant as working definitions only, however, and more operational definitions are needed when it comes to practical experimentation. For instance, it is impossible to determine the bioavailability of a compound "at any given time", as any bioavailability-sensing device must be permitted time (minutes) to interact with the analyte.

For measurement of bioavailable ammonium, we have long had chemical methods that allow for measurement of ammonium following a range of nonexhaustive extraction procedures (*3, 4*). More advanced methods relying on measurement of the chemical activity of ammonium include ion-specific electrodes (*5*), oxygen microelectrodes with build-in signal-transducing bacteria (*6*), ammonia gas sensors (*7*), and optodes (*8, 9*). However, none of these methods allow for estimation of bioavailable ammonium associated with soil or sediment particles. In addition, they are often limited by relatively poor sensitivity and by interference from other compounds present in the soil-pore water solution.

In the absence of biological techniques capable of directly measuring ammonium bioavailability, it is impossible to validate the above-mentioned chemical methods for their ability to quantify the bioavailable fraction of ammonium in soil. Recombinant whole-cell bacterial biosensors have been used to estimate bioavailability of both dissolved and particle-associated pollutants in soil (*10, 11*) and may provide one solution to this problem. We here present a novel biosensor approach based on the lithoautotrophic ammonia-oxidizing bacterium *Nitrosomonas europaea* transformed with a sensor plasmid containing the *luxAB* genes from *Vibrio harveyi* (*12*). In this strain bioluminescence (i.e., luciferase activity) is tightly coupled to ammonia oxidation activity. This linkage has previously been exploited in several studies to monitor toxic compounds in wastewater (*12*), soil (*10*), and root-exudate extracts (*13, 14*). Our present study represents a novel application of the strain to accurately and rapidly quantify bioavailable pools of ammonium in soil.

Experimental Section

Bacterial Biosensor Strain and Growth Conditions. The bioluminescent *N. europaea* ATCC 19718 (pHLUX20) reporter strain was used for all experiments (*12*). The strain constitutively expresses the *luxAB* genes from *Vibrio harveyi*. The biosensor strain was routinely grown at 28 °C in autotrophic growth medium (AG medium, pH 7.5) containing kanamycin (25 mg L^{-1}) as described previously (*10, 15*).

Ammonium Biosensor Assay in Ammonium Standard Solutions. Early stationary-phase *N. europaea* biosensor cultures (6×10^7 cells mL^{-1}) were harvested by centrifugation (10000 *g*, 4 °C, 10 min) and resuspended in fresh AG medium without ammonium sulfate (pH 7.5, 7.8, or 8.0). In order to test biosensor performance at different cell densities, the resulting cell pellets were resuspended in 10, 25, or 100% of the original volume. Resting biosensor cell suspensions were

* Corresponding author e-mail: kkb@life.ku.dk.
† University of Copenhagen.
‡ National Environmental Research Institute, University of Aarhus.
§ Institute of Biological Science, University of Aarhus.

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kept at 22 °C for up to one h before use without affecting results (data not shown). The chosen centrifugation protocol and medium composition for the biosensor assay were based on results from optimization experiments (see Supporting Information Appendix 1). Cell densities were determined as described previously (*15*).

Biosensor incubations were initiated by mixing aliquots of 0.5 mL ammonium-starved resting biosensor suspension with 0.5 mL ammonium sulfate solutions at appropriate concentrations in 2-mL transparent polypropylene microtubes. Incubations were carried out on a rotary shaker (200 rpm) for 10, 30, or 90 min at 22 °C in the dark. Finally, bioluminescence was measured by luminometry (1253 luminometer, BioOrbit, Turku, Finland). Fifty μL of luciferase substrate solution (35 μL of *n*-decanal dissolved in 250 μL of dimethyl sulfoxide diluted in 9.75 mL of deionized water; Milli Q system, Millipore, Bedford, MA, USA) was added to biosensor/sample suspensions 5 s prior to bioluminescence measurement (see Supporting Information Appendix 2 for optimization of *n*-decanal delivery). All bioluminescence values are given as the mean of two analytical replicates.

Soil Microcosm Set Up. An alluvial rice paddy soil from the Mekong delta previously characterized in detail (*16*) was used for *in situ* biosensor assays. The soil (0−15 cm depth) was air-dried (~30 °C), sieved (2-mm mesh size), and stored at 5 °C prior to use.

Aerobic incubation experiments were set up with 100 g of soil in 250-mL Erlenmeyer flasks at 22 °C in the dark. Following preincubation for 24 h different doses of urea were sprayed into the soil while increasing soil moisture to about 30% (wt/dry wt) corresponding to 60% of the water-holding capacity. Urea rather than ammonium salt was used to provide more even distribution of soil ammonium (*17*) and is often used as N fertilizer in rice production (*18*).

Anaerobic incubation experiments were set up in microcosms with 0.5 g of soil in 50-mL glass vials. This soil was saturated by adding 0.5 mL of deionized water to simulate rice paddy conditions. Subsequently, the vials were tightly capped with gastight rubber stoppers and the head space was flushed with N_2 for 2 min. Following preincubation for 4 d at 22 °C in the dark, 0.2 mL of water or urea solution was injected into the vials to obtain doses of 0 and 51 μg urea g^{-1} dry wt soil, respectively. Both oxic and anoxic microcosms were sampled at regular intervals for up to 8 d to measure bioavailable and extractable ammonium pools (see below).

Biosensor Assays for Determination of Bioavailable Ammonium in Soil. Biosensor assays for bioavailable ammonium were performed using both a soil−water supernatant assay for determination of bioavailable ammonium in soil-derived aqueous extracts and a solid phase-contact assay allowing for direct interaction of biosensor bacteria with particle-associated ammonium.

Soil−water supernatant assays using soil incubated under aerobic conditions were prepared by mixing 0.5 g of urea-amended soil with 5 mL of deionized water in 9-mL polyethylene tubes. The tubes were incubated horizontally on a rotary shaker (250 rpm, 22 °C) for 60 min, and soil−water supernatants were collected by centrifugation (10000 × g, 10 min, 22 °C). Soil−water supernatants from soil incubated under anaerobic conditions were collected in the same way except that no water was added prior to centrifugation. Following centrifugation, aliquots of 0.5 mL soil−water supernatant were transferred into 2-mL transparent polypropylene microtubes and mixed with 0.5 mL of resting biosensor cell suspension (1.2×10^8 cells mL^{-1}, pH 7.8). The resulting suspension was initially mixed vigorously before incubation on a rotary shaker (250 rpm; 22 °C) for 10 min in the dark. Bioluminescence was recorded as described above for ammonium standard solutions except that all bioluminescence values are given as the mean of four analytical replicates.

Solid phase-contact assays were performed in soil−water slurries (ratio of 1:10 wt/wt). For oxic soil samples, 0.5-g soil samples were initially transferred to 9-mL polyethylene tubes (Ole Dich, Hvidovre, Denmark), and soil pH was adjusted to 7.8 by adding a predetermined amount of 1 M KOH. Subsequently, 5 mL of resting biosensor cell suspension (3.0×10^8 cells mL^{-1}, pH 7.8) was added followed by vigorous mixing and incubation on a rotary shaker (250 rpm; 22 °C) for 10 min in the dark. After incubation, aliquots of 1 mL suspension were transferred into 2-mL transparent polypropylene microtubes, and bioluminescence was recorded as described above with four analytical replicates. Anoxic samples were treated and measured in the same way except that pH adjustment (pH 7.8) and biosensor incubation took place directly in the 50-mL glass vials used for microcosm incubation. The large headspace and the vigorous mixing ensured oxygenation of the sample allowing for assessment of the O_2-dependent bioluminescence reaction.

Ratiometric Normalization Procedure for Estimation of Bioavailable Ammonium in Soil. Direct measurement of bioluminescence in soil slurries suffers from interference due to masking of emitted light by suspended soil particles (*10, 11*). Hence, it is impossible to directly link bioluminescence recordings in soil slurries to bioluminescence recordings in particle-free standard solutions. In order to circumvent this problem we employed a ratiometric normalization procedure taking advantage of the broad ammonium concentration range (10−100 mM) for maximal biosensor activity (see Supporting Information, Figure S1). For each sample containing unknown levels of ammonium, the bioluminescence was recorded as the percentage of maximum bioluminescence ($RLU_{\%max}$) using the following formula

$$RLU_{\%max} = \frac{RLU_{sample}}{RLU_{max}} \times 100\% \quad (1)$$

where RLU_{sample} equals bioluminescence in the tested sample without ammonium amendment and RLU_{max} equals bioluminescence in the tested samples amended with 33 mM NH_4^+. Bioluminescence data from external ammonium standard solutions were treated in the same way and presented as $RLU_{\%max}$ as a function of known ammonium concentrations in standard solutions. Using this ratiometric normalization procedure, we estimated bioavailable ammonium in both supernatants (soil−water supernatant assay) and slurries (solid phase-contact assay) with reference to the ammonium concentration in external standard solutions.

Chemical Analysis. Total dissolved ammonium was determined by spectrophotometry (*19*). Exchangeable ammonium was determined in 2 M KCl-extracts using the same extraction conditions as for the soil−water supernatants. Soil extracts were frozen (−18 °C) prior to analysis.

Results and Discussion

Optimization of Ammonium Biosensor Performance in Ammonium Standard Solutions. The response of bioluminescence to ammonium was linear for concentrations up to 1000 μM NH_4^+. The analytical (i.e., linear) range decreased with increasing pH, whereas biosensor sensitivity increased with increasing pH (Figure 1 and Supporting Information, Table S2). This trend is consistent with the preferential uptake of ammonia rather than ionic ammonium in *N. europaea* (*20*). The biosensor did not respond to urea when tested in the 0.01−100 mM range consistent with the lack of urease activity in *N. europaea* (*21, 22*). Further, the biosensor did not respond to any of the 20 amino acids that are used for

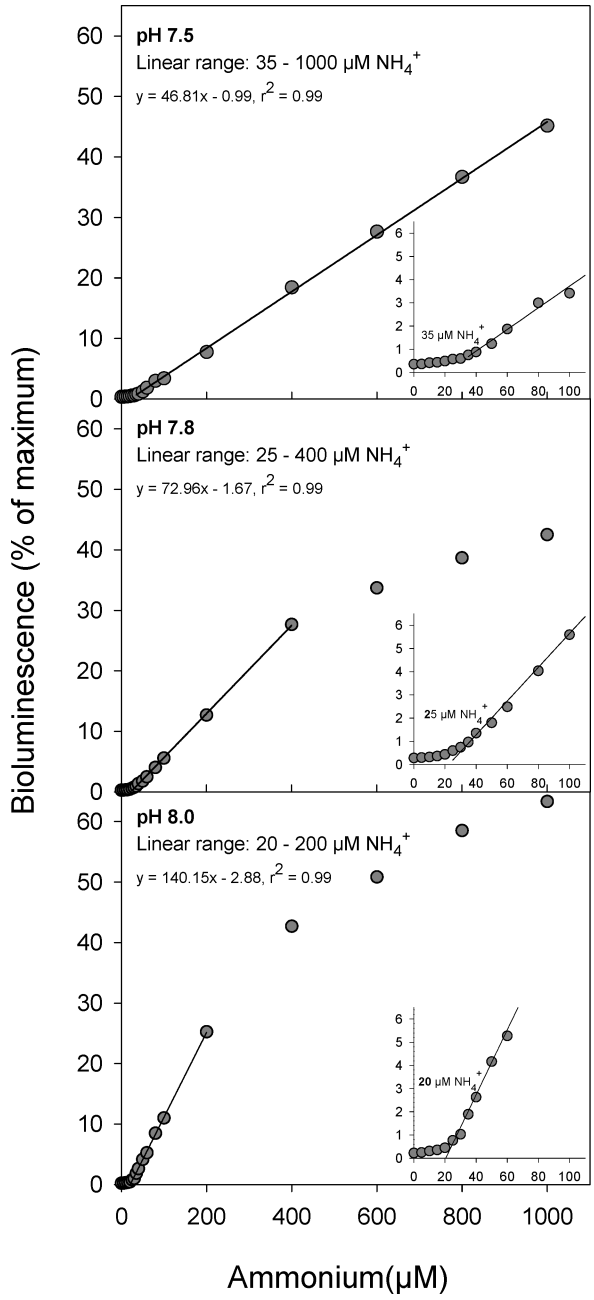

FIGURE 1. Representative calibration curves for *Nitrosomonas europaea* ammonium biosensor (3.0×10^8 cells mL^{-1}, 10 min incubation time) at pH 7.5 (upper panel), 7.8 (middle panel), and 8.0 (lower panel). Maximum bioluminescence was obtained with 33 mM ammonium. Inserts show enlargements of the biosensor responses between 0 and 100 μM ammonium. Concentrations of ammonium equal the sum of NH_4^+ and NH_3. Standard errors ($n = 2$) for each bioluminescence measurement averaged 1.5, 2.5, and 1.4% of average bioluminescence values at pH 7.5, 7.8, and 8.0, respectively.

protein synthesis in living organisms when tested at a concentration of 100 μM.

Decreasing the incubation time from 90 to 10 min reduced the quantification limit of the assay (e.g., from 88 μM to 22 μM with 3.0×10^8 cells mL^{-1} at pH 7.8) but reduced also the upper limit for ammonium quantification (e.g., from 800 to 100 μM with 3.0×10^8 cells mL^{-1} at pH 7.8). At short incubation times (e.g., 10 min; pH 7.5), biosensor sensitivity increased with increasing cell density, whereas the opposite trend was evident at long incubation times (e.g., 90 min; pH 7.5). These trends may be explained by increased analyte consumption

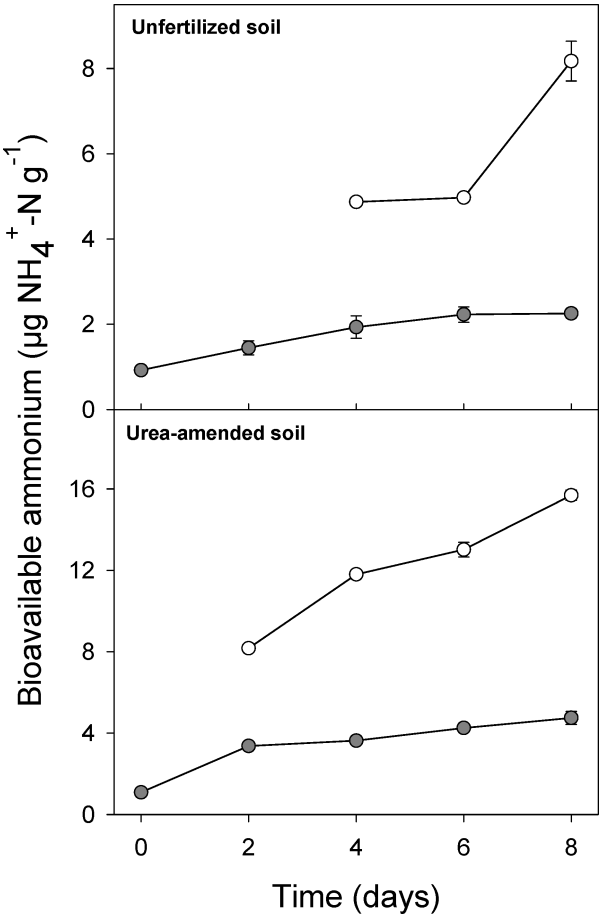

FIGURE 2. Bioavailable ammonium (sum of NH_4^+ and NH_3) as determined by soil−water supernatant biosensor assay (1.2×10^8 cells mL^{-1}, pH 7.8, 10 min incubation time) in soil−water supernatants derived from oxic (○) and anoxic (●) soil samples. See text for details on differential extraction protocols used for oxic and anoxic soil samples. First soil sampling was performed one hour after urea amendment. Means ± one standard error ($n = 2$) are shown.

with increased incubation time and biosensor cell density leading to decreased cell-specific bioluminescence over time.

As a compromise between optimal biosensor sensitivity and analytical range for ammonium quantification all subsequent experiments were performed as short-term incubations (10 min) at pH 7.8 using either an intermediate or high cell density (i.e., 1.2×10^8 or 3.0×10^8 cells mL^{-1}). Standard curves were always reproducible with only minor differences between different biosensor suspensions at different dates (see Supporting Information, Table S2).

Ammonium Bioavailability in Soil Solutions (Soil−Water Supernatant Assay). Bioavailable ammonium could be detected in soil solutions derived from both oxic and anoxic soil microcosms (Figure 2). Bioavailable ammonium increased to higher levels over time in urea-amended soil than in unfertilized soil (*23*). The effective ammonium quantification limit per g of soil was lower for anoxic than for oxic soil samples due to the different sample preparation procedures used for nonsaturated (oxic) and irrigated (anoxic) soils. Hence, ammonium levels could be determined at all times in samples derived from unfertilized, anoxic soil microcosms (Figure 2, upper panel), whereas ammonium levels were initially below the biosensor quantification limit in samples derived from oxic soil microcosms. This analytical limitation could be circumvented by analyzing soil−water supernatants derived from less diluted soil slurries with soil:liquid ratios of up to 1:5 (data not shown).

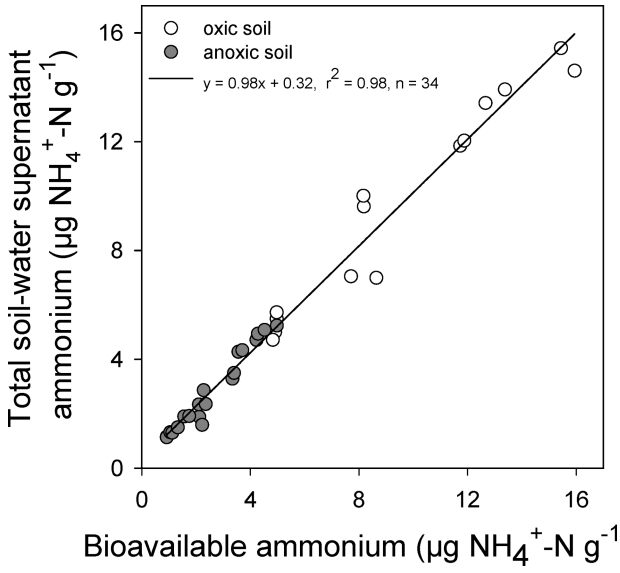

FIGURE 3. Correlations between different ammonium availability descriptors based on analysis of soil−water supernatant samples from individual oxic and anoxic soil microcosms: total soil−water supernatant ammonium (chemical assay) versus bioavailable ammonium (soil−water supernatant biosensor assay; 1.2 × 10^8 cells mL^{-1}, pH 7.8, 10 min incubation time). Concentrations of ammonium equal the sum of NH_4^+ and NH_3.

Biosensor and chemical quantification of ammonium gave highly comparable results as demonstrated by a near 1:1 correlation between ammonium concentrations estimated by biosensor and chemical methods, respectively (Figure 3). This demonstrates that all soil solution ammonium was bioavailable, as expected, and further validate the capability of the biosensor approach to accurately estimate ammonium concentrations in samples derived from both oxic and anoxic soil. Hence, by carefully controlling other key factors that affect metabolic activity (e.g., pH, oxygen availability, and temperature) it was possible to obtain accurate biosensor quantification of ammonium in soil-derived solutions.

Ammonium Bioavailability in Soil Slurries (Solid Phase-Contact Assay). Bioavailable ammonium increased to higher levels over time in urea-amended soil than in unfertilized soil (Figure 4). Bioavailable ammonium could be quantified irrespective of soil conditions even though low bioluminescence values were obtained due to masking of emitted light by soil particles (*10*). Hence, it was necessary to use high biosensor cell densities (3 × 10^8 cells mL^{-1}) and optimal pH (7.8) to consistently obtain high-quality bioluminescence measurements, defined as bioluminescence readings at least 10 times higher than background luminometer readings. Importantly, any differences of light-masking properties between different soil samples could be adequately compensated for by the ratiometric normalization procedure.

Bioavailable ammonium was correlated to water- and KCl-exchangeable ammonium in both oxic and anoxic soil (Figure 5) although correlations were somewhat weaker than observed for soil-derived water samples (Figure 3). The ratio of bioavailable to water-extractable ammonium averaged 2.8 for oxic soils and 13.0 for anoxic soils. These ratios were significantly higher than 1 (paired *t* tests, $P < 0.001$) and thus demonstrate that particle-associated ammonium contributed to ammonium bioavailability in soil, especially under anoxic soil conditions. Based on the conservative assumption that none of the water-extractable ammonium was initially associated with soil particles, we can estimate that at least 74% and 93% of the bioavailable ammonium was associated with soil particles in the oxic and anoxic soils, respectively.

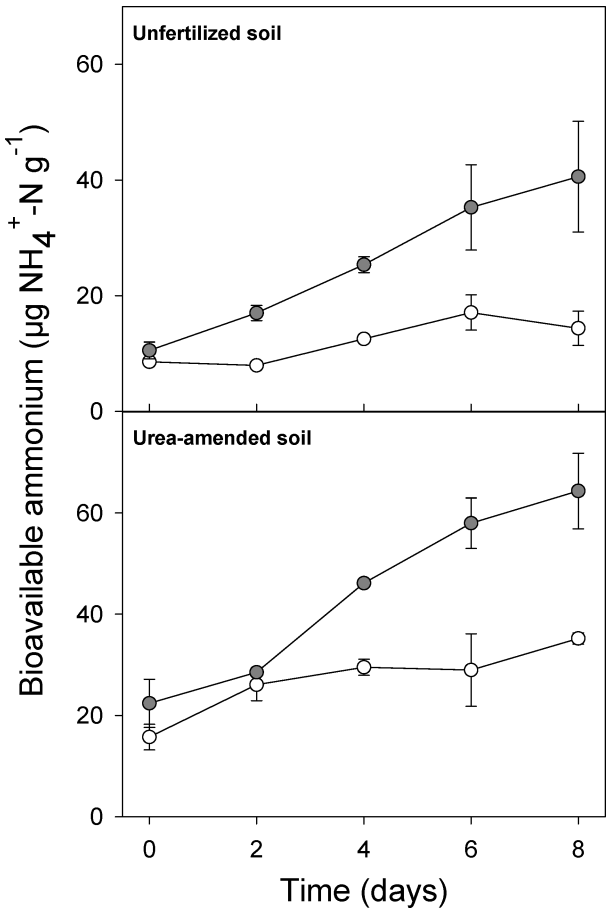

FIGURE 4. Concentrations of bioavailable ammonium (sum of NH_4^+ and NH_3) as determined by solid phase-contact biosensor assay (3.0 × 10^8 cells mL^{-1}, pH 7.8, 10 min incubation time) in soil slurries derived from oxic (○) and anoxic (●) soil samples. First soil sampling was performed one hour after urea amendment. Means ± one standard error ($n = 2$) are shown.

It is well-known that soil particle-associated ammonium including recently fixed ammonium contributes to the bioaccessible pool of ammonium for both plants and microorganisms (*3, 17, 24*). However, our study is the first to demonstrate that particle-associated ammonium also contributes significantly to the bioavailable pool of ammonium as defined operationally by the ability of soil particle-associated ammonium to stimulate bioluminescence emission of introduced biosensor bacteria. Still, we cannot conclude whether sorbed ammonium can be directly taken up by particle-associated biosensor cells or whether sorbed ammonium must briefly (<10 min) desorb from soil particles prior to bacterial uptake.

The average ratio of bioavailable to KCl-exchangeable ammonium was significantly higher for anoxic (1.13) than for oxic soils (0.77) demonstrating that anoxia promoted a higher bioavailability of ammonium (unpaired *t* test, log-transformed data, $P < 0.001$). Concentrations of bioavailable ammonium were significantly different than corresponding concentrations of KCl-exchangeable ammonium for oxic soils (paired *t* test, $P < 0.001$) but not for anoxic soils (paired *t* test, $P = 0.099$). However, the statistical power of the test for anoxic soil (0.256) was lower than the desired power (0.8). Combined with the low P-value obtained, we thus cannot exclude the possibility that bioavailable levels of ammonium exceeded KCl-exchangeable levels under anoxic soil conditions and that fixed ammonium therefore must have contributed slightly to the bioavailable pool of ammonium. The average ratio of bioavailable to extractable ammonium did not differ

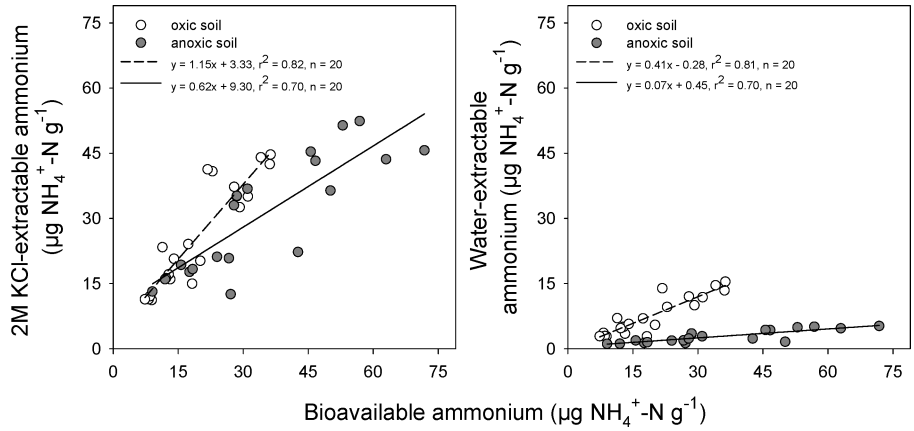

FIGURE 5. Correlations between different ammonium availability descriptors based on analysis of samples from individual oxic and anoxic soil microcosms. Left panel: 2 M KCl-extractable ammonium (chemical assay) versus bioavailable ammonium (solid-phase contact biosensor assay). Right panel: water-extractable ammonium (chemical assay) versus bioavailable ammonium (solid phase-contact biosensor assay). Biosensor assay conditions: 3.0 $\times$ 10^8 cells mL^{-1}, pH 7.8, 10 min incubation time. Concentrations of ammonium equal the sum of NH_4^+ and NH_3.

significantly between unfertilized and urea-fertilized soils in either anoxic or oxic soils (unpaired *t* tests, log-transformed data, $P = 0.39$ and $P = 0.27$, respectively).

KCl-exhangeable ammonium significantly overestimated bioavailable ammonium in oxic soils, which strongly suggests that nonexchangeable (i.e., fixed) ammonium did not contribute to bioavailability. By contrast, we cannot exclude the possibility that fixed ammonium may contribute to the bioavailable pool of ammonium in anoxic soils. Interestingly, such a contribution would be consistent with previous studies demonstrating high bioaccessibility of recently fixed ammonium pools to both ammonia oxidizers and plants under reduced soil conditions (*17, 25–27*) most likely due to decreased coating of clay minerals with Fe(III)oxides (*28, 29*).

Factors Interfering with Biosensor Performance. Sample matrix interference is a common problem when working with fluorescent or luminescent biosensors in soil. We therefore introduced the simple ratiometric normalization procedure based on bioluminescence measurement in parallel unamended and ammonium-amended soil samples to correct for soil masking of emitted light. Although this procedure may be considered less advanced than the use of double-tagged biosensors (*30*), it is comparable to the frequently used approach of using dual biosensor strains constructed in the same host strain for parallel analysis of soil samples (*31*).

Our data demonstrated that it is possible to use an unspecific biosensor strain (i.e., a metabolic activity bioreporter) for specific detection of ammonium provided that strict control of assay pH, oxygen availability, and temperature is maintained. The most significant limitation of the biosensor performance relates to the need for sample pH adjustment, which may lead to an overestimation of bioavailable ammonium in acid soils due to pH-dependent release of sorbed ammonium as volatile ammonia. However, even if chemical equilibrium is obtained during the short-term (10 min) biosensor assay (pH 7.8), only 3.5% of the soil ammonium (i.e., ammonium + ammonia; $pK_a = 9.24$; 25 °C) would be on the volatile ammonia form. As stated above, 77% of the KCl-extractable pool was bioavailable in the oxic soil. Assuming that fixed ammonium did not contribute to bioavailable ammonium in the oxic soil (see above) and thus to release of free ammonia, the ammonia released from acid soil particles during the 10 min of biosensor incubation will thus account to less than 5% of the bioavailable pool size estimated by the solid phase-contact biosensor assay. We therefore conclude that pH-dependent release of ammonia must have played only a minor role for the obtained results.

Sample toxicity is another factor that may interfere with biosensor performance, but this is not likely to constitute an important problem for the majority of soil samples due to the employed ratiometric normalization procedure and the short contact time (10 min) between sample and biosensor cells. Further, the use of EDTA in the biosensor medium will protect biosensor cells against toxicity from cationic metals (*32*). Lack of sample toxicity for most agricultural soils is supported by previous studies of linear alkylbenzene sulfonate (LAS) and copper toxicity in soil using the *N. europaea* biosensor (*10, 33*). Although most agricultural soils provide adequate conditions for activity of ammonia-oxidizing bacteria, it is possible that our biosensor assay will not work in soils deliberately amended with nitrification inhibitors or used for cultivation of certain crops extruding high doses of nitrification inhibitors from their roots (*13*). We therefore recommend that biosensor specificity and accuracy for ammonium quantification is routinely validated against chemical methods as exemplified in Figure 3.

NaCl amendment (500 mM) above the physiological limit for *N. europaea* growth did not markedly affect biosensor bioluminescence at high ammonium concentrations but strongly inhibited biosensor performance at low concentrations (see Supporting Information, Figure S4). However, lower salinities within the physiological limits for optimal growth (i.e., 0−100 mM NaCl (*34*)) are unlikely to affect biosensor performance although we recommend to grow biosensor cells at the salinity of the analytical samples.

Comparison with Alternative Techniques for Determination of Bioavailable Ammonium in Soil. The developed solid phase-contact biosensor assay is unique by its ability to interact with exchangeable and fixed pools of ammonium and by its ability to quantify bioavailable ammonium levels in soil. Previous attempts to use whole-cell bacterial biosensors have relied on bioluminescent N starvation biosensors constructed in *Pseudomonas fluorescens* (*35–38*). However, the N starvation biosensors are mainly useful for autecology studies focusing on the physiological status of bacteria introduced into soil, and it has proven difficult to use them for quantitative determination of bioavailable N in soil (K. K. Brandt, unpublished data). Hence, N starvation biosensors will work only in samples showing N limitation and even then the sensor cells will respond only to altered N levels following extensive bacterial growth resulting in depletion of bioavailable soil N. By contrast, our *N. europaea* biosensor assay allows for rapid and sensitive quantification of bioavailable ammonium following just 10 min of incubation in the sample. Further, the *N. europaea* biosensor is

self-reproducible, can easily be stored by standard microbiological preservation methods, and therefore represents a cheap analytical method.

Perspectives for Use of Ammonium Biosensors in Environmental Monitoring. The developed *N. europaea* biosensor assay for determination of bioavailable ammonium should have broad relevance for soil fertility testing and may possibly even work as a proxy for quantification of N bioavailability to crop plants in soils, where ammonium is the predominant inorganic N species. Specifically, we propose that the biosensor should be ideal for future investigations of soil fertility in irrigated rice soils that are characterized by steep redox gradients in both time and space (*39*), likely to result in dynamic spatiotemporal changes of ammonium bioavailability. Further, the developed solid phase-contact assay may be easily adapted to other sample matrices such as sediments and nitrifying sludges, where ammonia oxidizers have crucial functions and ammonium bioavailability has utmost importance for ecosystem function.

The selected *N. europaea* biosensor is inherently limited by the physiological requirements of the strain with regard to ammonium uptake affinity, optimal salinity range, pH, etc. However, more sensitive quantification of ammonium may be possible following construction of new biosensors in more oligotrophic ammonia oxidizer strains (*21*). Likewise, biosensor analysis in marine and estuarine samples may be obtained by using halophilic or halotolerant host strains for biosensor construction (*21*).

In conclusion, we have successfully developed and applied novel whole-cell bacterial biosensor assays for rapid and robust quantification of bioavailable ammonium in unfertilized and fertilized soils. Our results demonstrate markedly higher bioavailability of particle-associated ammonium in anoxic soil as compared to oxic soil. Future biosensor optimization should seek to optimize biosensor performance across a variety of sample types such as different soil types, sediments, etc.

Acknowledgments

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Supporting Information Available

Detailed descriptions of biosensor assay optimization experiments: bioluminescence protocol, biosensor cell preparation, and medium composition. Differences of chemical composition between tested autotrophic growth media (Table S1). Overview of results obtained for biosensor performance as affected by cell density, pH, and incubation time (Table S2). Impact of number of cell washing steps on biosensor performance (Figure S1). Impact of biosensor cell resuspension medium for biosensor performance (Figure S2). Impact of organic solvents used for *n*-decanal delivery on bioluminescence of biosensor cells (Figure S3). Impact of NaCl on biosensor performance (Figure S4). This material is available free of charge via the Internet at http://pubs.acs.org.

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