Optical Chemical Sensors for Soil Analysis: Possibilities and Challenges of Visualizing NH_3 Concentrations as well as pH and O_2 Microscale Heterogeneity

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ABSTRACT: Agricultural nitrogen (N) application to soils is the main source of atmospheric ammonia (NH_3) emissions. Ammonia negatively impacts the environment on a large scale. These emissions are affected by spatiotemporal heterogeneities of parameters within the soil on a microscale. Some key parameters controlling processes of the N cycle are soil oxygen (O_2) and pH. To better understand biogeochemical soil processes and NH_3 emissions we propose the application of optical chemical sensors (optodes) in soils. The use of optodes in soil science is in its infancy. In this study, we investigated the possibilities and challenges of using optodes in non-waterlogged soils with the extended application of a recently developed NH_3 optode in combination with pH and O_2 optodes in two different soils and with different fertilizers. Our results demonstrated the possibility to visualize reductions of NH_3 concentrations by 76 % and 87 % from the incorporation of sludge compared to the surface application of sludge. We showed in 2D how soil pH and fertilizer composition correlate with NH_3 volatilization. Our measurements revealed that pH optodes show certain advantages over conventional methods when measuring pH in soils *in-situ*. Lastly, we investigated spatiotemporal dynamics of O_2 at

different soil water contents and discussed potential challenges, which can lead to measuring artifacts.

INTRODUCTION

Soils facilitate life and feed the world population as they are the fundament for healthy ecosystems and food production[1]. Soils are complex biological systems due to their high biogeochemical activity and spatiotemporal heterogeneities. Distinct physical, chemical, and biological soil properties create microsites within the soil matrix. These sites are involved in important soil processes including both nutrient cycling and gas formation, as they are highly influenced by soil heterogeneity[2–5].

In agricultural soils, fertilization and soil amendments affect the soil composition as well as soil properties. Different fertilizer management strategies may cause variations of soil pH, substrate availability, and O_2 within the soil matrix. Within the soil, the production and formation of ammonia (NH_3) are highly sensitive to changes in soil parameters such as soil moisture, pH, O_2, and different nitrogen (N) forms[5,6]. During the last century, N fertilizers have been applied excessively to agricultural soils, thereby increasing the emissions of reactive N gases (e.g., NH_3 and nitrous oxide (N_2O))[7]. Ammonia emissions from agriculture accounted for 96 % of the European atmospheric NH_3 release, partly due to the low efficiency of fertilizer uptake[8]. Ammonia emissions pose an environmental risk through N deposition, acidification, and eutrophication[9]. Furthermore, they contribute to the formation of atmospheric particulate matter ($PM_{2.5}$), which is associated with adverse health effects[10,11]. Thus, there is a great demand to mitigate NH_3 emissions.

To improve mitigation strategies, a detailed insight into local processes and interactions of soil and fertilizers at microscale is needed, as this could explain the great variabilities seen in NH_3 emission factors[12]. Therefore, it is important to monitor concentrations of emitted NH_3 at soil/air and soil/fertilizer interfaces, and at the same time continuously measure spatiotemporal changes of important soil parameters in distinct microsites as these can provide a deeper understanding of NH_3 emission dynamics. To date, the general approach to study soil processes and gas emissions relies on bulk measurements of soil compounds and gas concentrations. These bulk measurements fail to provide the spatial and temporal resolution, especially at the mentioned interfaces, needed for an in-depth understanding of these complex processes. Planar

optical sensors, also termed optodes[13,14], may provide the methodological platform for high-resolution spatiotemporal studies of soil processes.

Optodes are reversible optical sensors that enable monitoring of variations in analyte concentrations (e.g., O_2, pH, NH_3, CO_2) for several days with imaging intervals ranging from seconds to hours. Thus, optodes offer non-invasive and *in-situ* imaging of analytes at high spatial and temporal resolution. In short, optodes show a change in photoluminescence after interacting with an analyte[15,16]. They consist of an analyte-sensitive luminophore, which is immobilized within a polymer matrix and coated onto a support material, such as a plastic foil. There are two possibilities for referenced readout, which are lifetime-based, and ratiometric imaging[14]. Optodes show great promise for studying soil biochemistry as they visualize analyte changes in real time and without sample pre-treatment.

Some optodes, such as for pH and O_2, are well-established tools to study complex environments, in particular sediments and waterlogged soils[17,18]. However, only a few studies applied optodes within non-waterlogged soils[19,20–26]. Most relevant soil processes, from an agricultural perspective, should be studied at lower water contents relevant for plant growth (40-90 % of the water holding capacity (WHC)). Optodes for NH_3[27–29] or NH_4^+ are mainly available for the detection of low ppb concentrations and are rarely used in soils. Therefore, they are still not well characterized. Strömberg *et al.* proposed an NH_4^+ optode that could be used in soils, but despite a few studies, this optode has not been applied since[25,30,31]. Recently, we developed a dedicated NH_3 optode working in a higher concentration range[20]. The new NH_3 optode is well suited for soil studies and it can be combined with other optodes to acquire complex spatiotemporal patterns in 2D.

The objective of this study was to identify the possibilities and challenges of using optodes in non-waterlogged soils and soils with different soil physico chemical characteristics. Therefore, optodes for NH_3, pH and O_2 were implemented in several laboratory soil experiments to assess their usability in different scenarios related to agricultural practices or natural events occurring within soils (Figure 1). For this, dairy processing sludge (DPS) was chosen as organic fertilizer. Dairy processing sludge is an emerging biobased fertilizer as it is an organic waste product rich in phosphorus (P) and N derived from the wastewater treatment of the dairy industry[32]. One of the reasons for its use is the goal of more sustainability for food and agricultural systems[33]. The soils used were two loamy sandy soils typical for Danish agricultural soils and differed in their pH values. Additionally, pH optodes were tested to measure pH in

non-waterlogged soils *in-situ* and O_2 optodes to test the usability of such at different soil water contents.

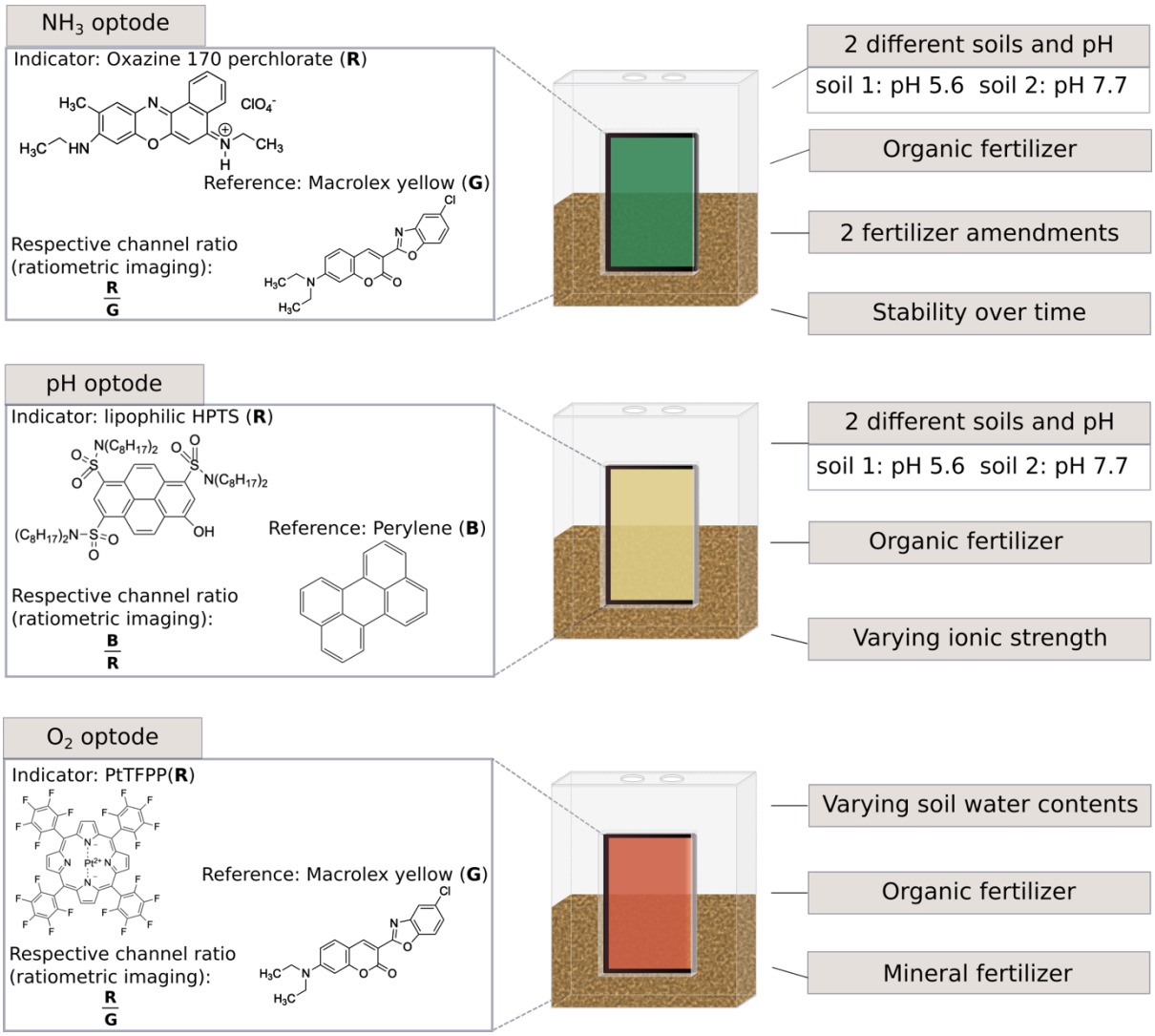

Figure 1. Overview of the experimental set-up and utilized optodes as well as the various test scenarios.

MATERIALS AND METHODS

Soil and dairy sludge. Two soils that differ in pH were used in the different optode studies (Table S1). Soil 1, a sandy loam with a high organic matter content was collected from 0 to 20 cm depth from an experimental field site at Aarhus University, Foulum, Denmark (56° 30' N, 9° 34'). The fresh soil was collected in late October 2020, passed through a 4-mm sieve, and stored in a cold room (4 °C) for two weeks until the implementation of the experiment. Soil 1 had a relatively low pH_{H2O} of 5.6 (Table S1). The second soil (Soil 2) was also a sandy loam with a naturally high content of calcium carbonate ($CaCO_3$) originating from the surrounding moraine. This soil was collected in February 2021 from an agricultural field located in Tølløse, Zealand, Denmark (55° 37' N, 11° 48'). After collection, the soil was passed through a 2-mm sieve and stored at 4 °C. This soil had a relatively high pH_{H2O} of 7.7 due to a high content of $CaCO_3$. The soil textures were characterized by AGROLAB Agrar/Umwelt (Sarstedt, Germany). Soil pH was measured in a 1:2.5 w/w soil/water ratio and with a micro glass pH-electrode (type 6.0234.110, Metrohm, Herisau, Switzerland) connected to a pH meter (type 764, Knick, Berlin, Germany) with a temperature probe calibrated using standard pH buffers (Fluka analytical). Further soil properties were analyzed and details can be found in the Supporting Information (SI). Soil properties of soil 1 and soil 2 are listed in Table S1.

DPS was obtained from a wastewater treatment plant of a dairy production factory in Videbæk, Denmark. It was stored at -18 °C until three days prior to the start of the experiment. The chemical properties of DPS were measured by an accredited laboratory (Højvang laboratorier A/S, Denmark). Properties of the DPS are presented in Table S1.

Experimental setup for NH_3, pH and O_2 measurements. A total of five studies were conducted to elucidate different aspects of implementing optodes in soil studies using different N fertilizers, optode combinations, soil types, and water contents. An overview of the different studies can be found in Table 1, and Figure 1. For the experimental setup specially designed transparent plastic chambers with removable front walls and lids were used as measurement chambers (L x W x H: 60 x 39 x 100 mm) (Figure 1 & Figure S2). Soil and fertilizers were incubated in the chambers, while optodes were fixed on an integrated glass window (50 x 50 mm) equipped on the front walls.

In all five studies (Table 1), the soils were packed into the chambers achieving a soil bulk density of 1.3 g cm^{-3} resembling field soil bulk density. The soil packing method was adopted from Zhu et al.[34] and Nguyen et al.[35]. In studies using Soil 1, the chambers were packed

to 36 mm depth, and in studies with Soil 2, the chambers were packed to 38 mm depth, thus both the soil and the air above could be investigated through the optode window. The gravimetric soil water contents in studies 1-4 were kept at 35 %, which corresponds to 80 % and 93 % of WHC for soils 1 and 2, respectively. This water content resembles moist non-waterlogged field soil. In all studies the chambers were closed on top with a lid to ensure the soil water content remained constant.

In Studies 1-4, DPS was applied either in the middle of the soil (SM) or on top of the soil (ST) to monitor the differences in NH_3 emissions, pH, and in one case O_2 from these two treatments applied on the two different soils. The middle layer with DPS was a hotspot of soil/sludge mixture where 5 % and 4.4 % (w/w) DPS were applied on a dry matter basis in soils 1 and 2, respectively. The amount of sludge mixed into the layer was chosen to make up the air-porosity volume in the soil equal to the other layers. The amounts of soil, sludge, and water used for each layer can be found in the Supporting Information (SI). In the ST treatments the same amount of DPS that was used to mix in the SM treatments was simply applied in a layer on top of the soil. Control chambers with no DPS amendment were included in all studies.

In order to investigate the difference in O_2 level at one constant gravimetric soil water content (35%) with DPS, one chamber was also equipped with an O_2 optode in study 1 using soil 1 and applying the sludge in the middle (SM). This was compared to study 5. In Study 5, the use of O_2 optodes under different gravimetric soil water contents relevant for plant growth was investigated. Three different gravimetric water contents of 18 %, 25 % and 32 % corresponding to 41 %, 57 % and 73 % of WHC designated as low (L), medium (M), and high (H) water content, respectively, were included. This investigation was included to describe the more general use of optodes under agricultural relevant water contents. The chambers were filled with soil 1 and packed in the same way as described above, however, varying amounts of water were added. Instead of sludge 750 mg of mineral fertilizer (calcium ammonium nitrate, CAN, Yara), equivalent to 101.25 mg NH_4-N, were distributed on top. Furthermore, rain was simulated by adding equal amounts of water to each chamber to raise the water contents by 11 %, which equals a 4.67 mm rain event. This altered the soil water contents to 29 % (Rain-L), 36 % (Rain-M), and 43 % (Rain-H), respectively.

Planar optode fabrication. Optodes for NH_3 were prepared as previously reported by Merl & Koren[20] and so were optodes for O_2 and pH[20,36]. A detailed description of the preparation steps can be found in the Supporting Information (SI).

Imaging setup and measurement. In studies 1 and 2 the imaging setup consisted of a SLR camera (EOS 1300D, Canon, Japan) combined with a macro-objective lens (Zoom lens EF-S18-55mm f/3.5-5.6 III, Canon, Japan), a yellow 455 nm long-pass filter (GG455 SCHOTT, 52 mm x 2 mm) with another plastic filter (#10 medium yellow; LEEfilters.com). The plastic filter was mounted in front of the long-pass filter to regulate background fluorescence. A 405 nm UV LED (r-s components, Copenhagen, Denmark) paired with a short-pass filter (Hoya B-390 HFB 3925, UQG Optics, Cambridge, England) was used to excite the optodes. The LED, which functions as the flashlight, was controlled with a trigger box. This box is a USB-controlled LED driver unit (imaging.fish-n-chips-de) and is operated by the Look@RGB (imaging.fish-n-chips-de) software, which also enables the gathering of the sample images and simultaneously operates the SLR camera and LED.

In Studies 3 and 4 the imaging setup differed in the SLR camera, which had the near-infrared filter removed (EOS 1300D, Canon, Japan), and a macro-objective lens (Macro 100 F2.8 D, Tokina, Japan), as well as an orange 530 nm long-pass filter (OG530 SCHOTT, 52 mm x 2 mm). Instead of an UV LED, a blue LED (470 nm) together with a short-pass filter (Dichroic blue filter CDB-2511, UQG Optics, Cambridge, England) was used (Figure S1).

Calibrations were conducted prior to each experiment using the exact same setup as consecutively used in the respective experiment. Calibrations and data analysis for NH_3, O_2 and pH optodes were conducted as described by Merl & Koren[20] and previous studies[36–38]. In terms of the NH_3 optodes, it is important to emphasize that these were wet calibrations. The measured ratios from the optodes were translated into ppm as a mass fraction from mg L^{-1} as a result of the wet calibration. In order to assess the relative differences in NH_3 concentrations between treatments, the wet calibration was applied to the gas phase measurements. This is the fastest and simplest procedure at this point.

In each study, images were taken every 10 minutes for the first hour, and then the interval was increased to an image every hour, then to an image every two hours, up to an image every three hours. In total images were acquired for a total of 21 hours for all studies except the studies using O_2 optodes, in these long-term imaging was conducted for 7 days and up to 18 days.

Table 1: Overview of Studies 1-5 using ammonia (NH_3), pH and oxygen (O_2) optodes. Properties of the soils and DPS can be found in Table S1. DPS: dairy processing sludge.

	Study 1	Study 2	Study 3	Study 4	Study 5
Soil	Soil 1	Soil 2	Soil 2	Soil 2	Soil 1
Soil pH	5.6	7.7	7.7	7.7	5.6
Fertilizer type	DPS	DPS	DPS	DPS	Mineral fertilizer
Fertilizer application	Middle	Middle	Top & middle	Top & middle	Top
Duration	21 h/ 18 d	21 h	21 h	21 h	7 d
Gravimetric water content (%)	35	35	35	35	18, 25, 32
% of WHC	80	93	93	93	41, 57, 73
Optodes	NH_3, pH/ O_2	pH	NH_3	NH_3	O_2

RESULTS AND DISCUSSION

In terms of agricultural practices, the main aim is to adjust fertilizer management in a way that most of the applied N is utilized by the crops and to mitigate N loss (e.g. via NH_3 or N_2Oemissions). Hence, NH_3 concentrations resulting from varying fertilizer amendments were investigated with NH_3 optodes to assess the optodes' applicability as a screening method. Due to the interdependencies of NH_3 emissions with soil O_2 and pH, we also tested optodes for these analytes in similar settings as those chosen for the NH_3 optodes. These settings comprised non-waterlogged soils and the same organic fertilizer (DPS). Due to the higher dry matter content of DPS compared to manure it was possible to keep the soil non-waterlogged while using an organic fertilizer. Below, we show the findings of the usability of optodes in agricultural settings and non-waterlogged soils.

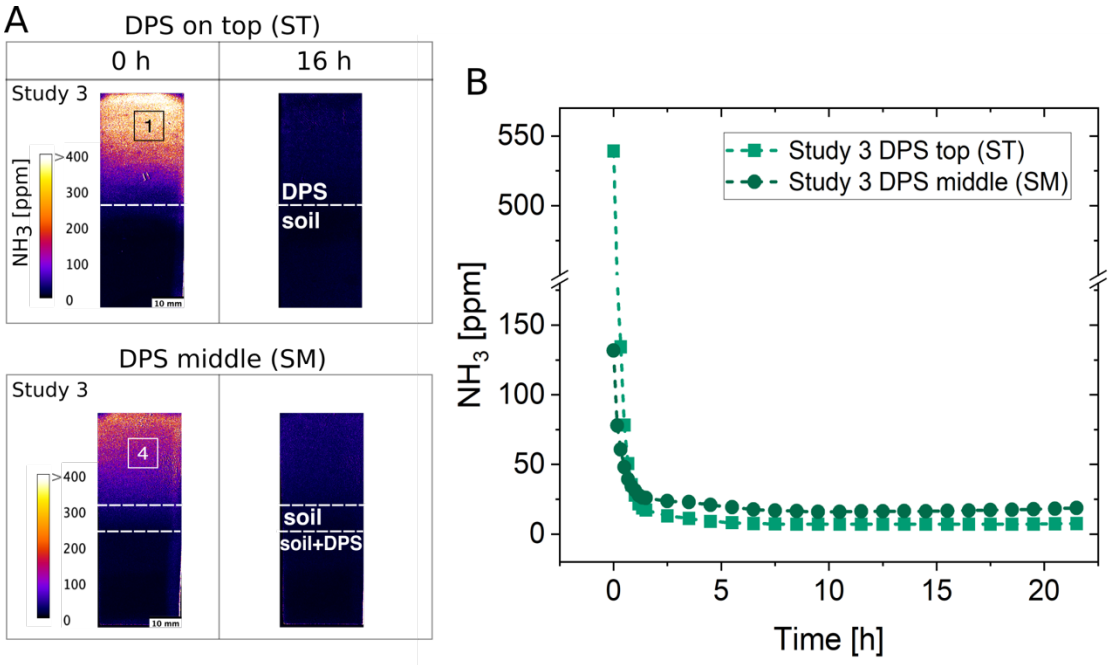

Figure 2. A: False color images showing the NH_3 concentrations of study 3 with the treatment organic fertilizer (DPS) on top (ST) and in the middle (SM). These false color images are examples from the start (0 h) and from a timepoint at 16 h. The dotted lines indicate where the soil and the mixture of soil and slurry interphase start. B: NH_3 concentrations from the regions of interest (ROIs) 1 and 4 as can be seen in A over a period of 20 h.

Ammonia optode performance in different soils and DPS application strategies. The possibility to monitor differences in NH_3 concentrations with optodes from dairy sludge (DPS) applied on top of the soil (ST) and from DPS incorporated into the middle of the soil (SM) was tested. This was done in Study 3 and 4 using soil 2 with pH 7.7 and adjusting the gravimetric soil water content to 35%. Examples of images and the concentration change over time are shown in Figure 2 for the NH_3 optode from Study 3. Regions of interest (ROIs) were chosen to represent the headspace above the soil and to illustrate differences in NH_3 dynamics. Higher NH_3 concentrations above the soil resulted from the ST treatment compared to the SM treatment. The results from Study 4 show the same pattern and can be found in the SI (Figure S5). In both treatments a sudden spike in NH_3 concentration can be seen immediately after sludge application. The NH_3 concentration was four times higher in the ST treatment than in the SM treatment in Study 3 and around seven times higher in Study 4 (Figure S5). Differences in the NH_3 concentrations between Study 3 and 4 could be due to a slight difference in the thawing and handling of the DPS in the experimental setup. In Study 4 the DPS was not completely thawed at the time of application, thus, infiltration differences of the sludge could have occurred as it is assumed that the more liquid (completely thawed) the fertilizer the faster the infiltration of such, resulting in lower NH_3 concentrations. The incorporation of sludge (SM) led to reductions of NH_3 concentrations by 76 % and 87 % in Studies 3 and 4, respectively, compared to the surface application of sludge (ST). These values of reduction in NH_3 concentration are within the range reported by Monaco *et al.* for a laboratory scale experiment investigating the NH_3 reduction from the incorporation of pig slurry compared to surface-applied slurry, which yielded a 81.7 % reduction in NH_3[39]. In field studies with the same objective reductions in NH_3 of 40-60 %[40] and 80 %[41] were reported. Additionally, the

immediate high NH_3 concentrations resulting from the surface application of fertilizer were also observed in laboratory scale experiments[39] as well as in field studies[40]. Upon the immediate NH_3 release a decline in NH_3 concentrations followed for about two hours. These concentration profiles over time were also seen in previous studies[20,39], and thus results from the NH_3 optodes follow the general expected trend. Lower NH_3 concentrations in the treatments with sludge applied in the middle are observed due to the soil creating a barrier, which induces a complete air-side resistance[42].

These NH_3 concentrations are not to be considered as absolute values but rather as a method to assess relative differences between treatments. There are two reasons for that. Firstly, we observed that the wet calibration is not directly applicable to the gas phase concentrations due to changes in humidities and because the newly developed NH_3 optodes are humidity dependent (e.g. the concentration measurements vary with humidity). The humidity dependency of the NH_3 optodes as well as the need to calibrate in the gas phase are not yet fully explored and need further investigations. Secondly, the chambers were kept closed while imaging NH_3 in the attempt to have well-defined soil system boundaries and thus eliminate the need to account for fluxes of energy and matter[1]. However, the emission of NH_3 is mainly restricted by the air-side resistance[42], therefore, chambers without a continuous air-flow will restrain the NH_3 emission due to an increased laminar film boundary while an increased gas-phase NH_3 concentration is obtained compared to natural field conditions[43].

The impact soil pH has on NH_3 concentrations is shown in Figure 3. In three studies sludge with a pH of 7.8 was applied into the middle of soils with different pH values. In Study 1 soil with a pH of 5.6 (Soil 1) and in Studies 3 and 4 soil with a pH of 7.7 (Soil 2) was used. The pH-dependent equilibrium of NH_3 and NH_4^+ causes NH_3-N to be mostly present as NH_4^+ (100%) at pH 6, whereas an increase to pH 8 results in a shift where both forms NH_4^+ (90%) and NH_3 (10%) are present (Figure 3A). This seemingly small shift in pH resulted in two and six times higher NH_3 concentrations above the soil in Study 3 and 4, respectively, compared to Study 1 (Figure 3B). The low pH soil (Soil 1) must have buffered the pH of the sludge, which was higher than the soil, and this resulted in lower NH_3 concentrations compared to the amendment of the same sludge in Soil 2. These results emphasize the importance of the chemical microenvironment of the soil on NH_3 emissions. Therefore, it can be an advantage to employ optodes for NH_3 and pH simultaneously as they can depict these interdependent and important processes further and on a high spatiotemporal scale.

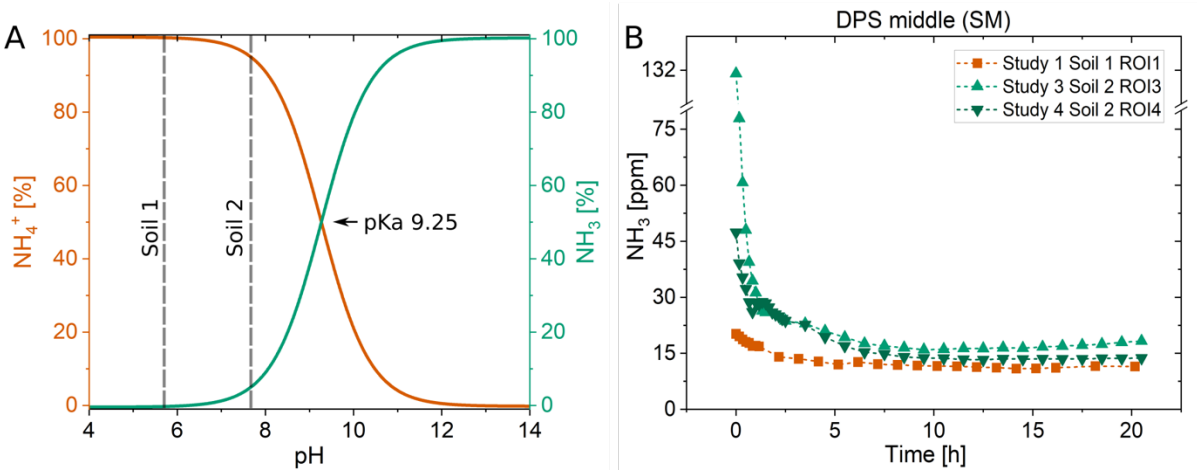

Figure 3. A: The pH-dependent equilibrium between ammonium (NH_4^+) and ammonia (NH_3) where the dotted lines show the pH the two soil samples (soil 1 and soil 2) had. B: NH_3 concentrations of the regions of interest (ROIs) taken from the air interphase above the soil from each study (study 1, 3 and 4) over a period of 20 hours in order to show NH_3 emission from soil 1 and soil 2 with the treatment (SM) organic fertilizer (dairy sludge) amended in the middle.

Using optodes to measure spatiotemporal variations in soil pH. Soil pH is a key parameter in soil fertility, as it controls redox reactions, nutrient and toxin bioavailability, and affects important biological processes[44,45]. This makes soil pH a parameter of general importance, and *in-situ* and constant monitoring will contribute to a better understanding of the complexity of soils. Traditionally, soil pH is assessed with conventional laboratory pH measuring methods utilizing potentiometric pH electrodes (in aqueous or mild saline extracts)[44]. While pH glass electrodes are fast and cover a wide pH range, they can only measure bulk pH in samples where the soil-to-solution ratio is changed to unnatural ratios[46]. Therefore, *in-situ* pH measurements at the soil's native gravimetric soil water content or spatially resolved measurements are not possible. This way hotspots of pH changes can not be determined in a complex system such as soil. Optical sensors for pH, on the other hand, can be used *in-situ*, without the need of sample extraction, and can therefore unravel the spatial and temporal pH heterogeneities of soils[44]. However, both methods have their limitations regarding precise pH measurements, which come from different non-thermodynamic assumptions inherent in their modes of operation[47]. Due to that, an important differentiation between optical pH sensing and potentiometric pH measurements needs to be considered, which is that optical pH sensors base pH measurements on the concentration of a pH-sensitive dye, whereas pH is measured as activity of hydrogen (H^+) ions in the potentiometric approach[46,47]. The latter is also known as the definition of pH in solution but soils can not always be in solution if the pH needs to be assessed in a non-waterlogged state. Soil ionic strength is another example that contributes to biased pH measurements and is a soil parameter of great importance known to have large variances[48]. Even though also optical pH sensors show cross-sensitivity to ionic strength the effect can be rather small[49]. A minimal effect of ionic strength on the response can be achieved, especially by using non-charged pH-sensitive dyes[49] as the one used in the pH optodes described in this study. The diminished impact different ionic strengths have on the used pH

optode is illustrated in Figure S6 and shows the slight differences in ratios at the same pH values but with three different IS (IS 0.10, 0.38 and 1.00 M).

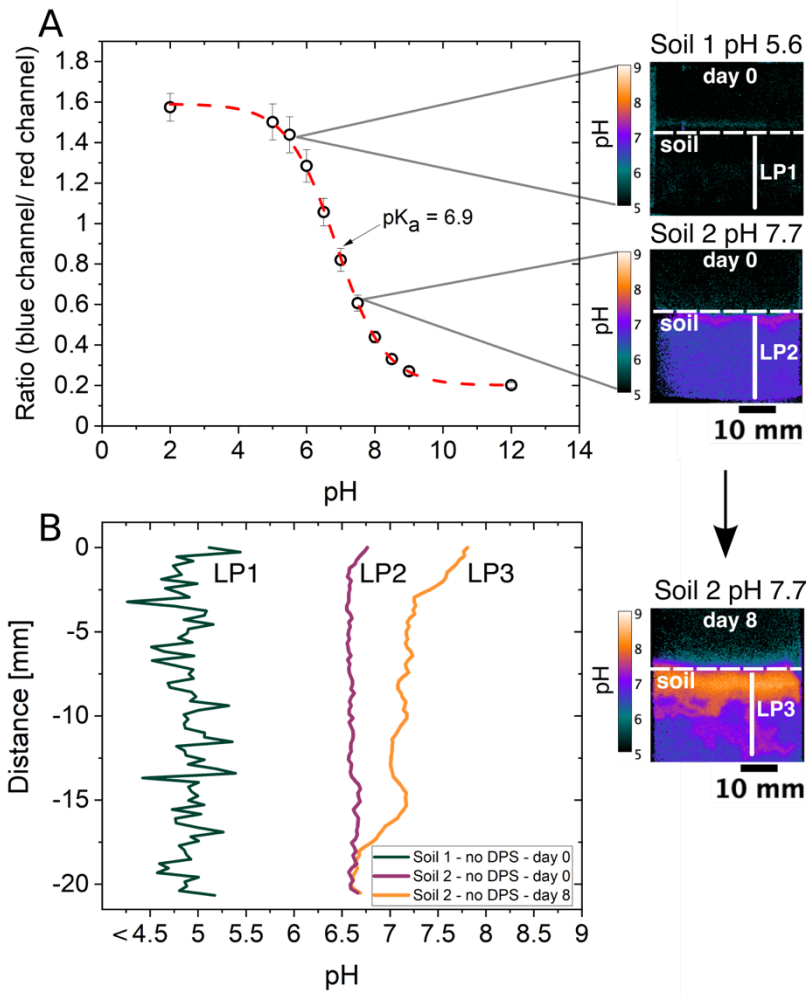

Figure 4. A: pH calibration curve showing the pK$_a$ of the respective pH optode and the dynamic range. Error bars depict the minima and maxima of the chosen regions of interest for each calibration step. B: Line profiles resulting from study 1 using soil 1 (pH 5.6) and study 2 using soil 2 (pH 7.7), where no sludge was added in either of the shown images.

Despite the advantages optical pH sensors bring to soil analysis they have not been used extensively in non-waterlogged soils so far. To expand beyond current approaches, we wanted to investigate the applicability of pH optodes in soils with lower soil water contents than in completely waterlogged soils, as waterlogged soils are not appropriate to study most relevant soil processes. We adapted the water content of both soils to 35 %, corresponding to 93 % of water holding capacity, which is a relevant water content when considering agricultural production. Here we show that pH optodes are indeed suited for soils with lower soil water content when considering some of the optical sensor's limitations. Figure 4A depicts a pH optode calibration curve with the dynamic range being between pH 5.5 to pH 8.5 and where the two soil samples' pH values are located within that range. While potentiometric pH sensors cover a wide pH range, optical pH sensors only cover a range of maximally 3 pH units, but their accuracy is superior to the potentiometric pH sensor within the sensitive range[46]. The highest

sensitivity of a pH-sensitive indicator dye is reached at pH = pK_a, and the limitation of the pH range is attributed to pH = pK_a ±1.5. Monitoring the pH works well in a non-waterlogged soil sample if the soil pH is well within the range of the sensor as seen in Figure 4A for Soil 2 but not so well if a soil sample is just at the edge of that range as seen for Soil 1 (Figure 4A). A reason for the possibility to measure pH even in samples that are non-waterlogged can be the polymer used to immobilize the pH indicator dye in. The optical sensor in this study was prepared with a hydrogel (Hydromed D4), which is known to have a water absorption characteristic of 50 %[50]. The polymer absorbs water present in the sample and swells, which is an advantage in facilitating proton exchange and detection even at relatively low soil water contents.

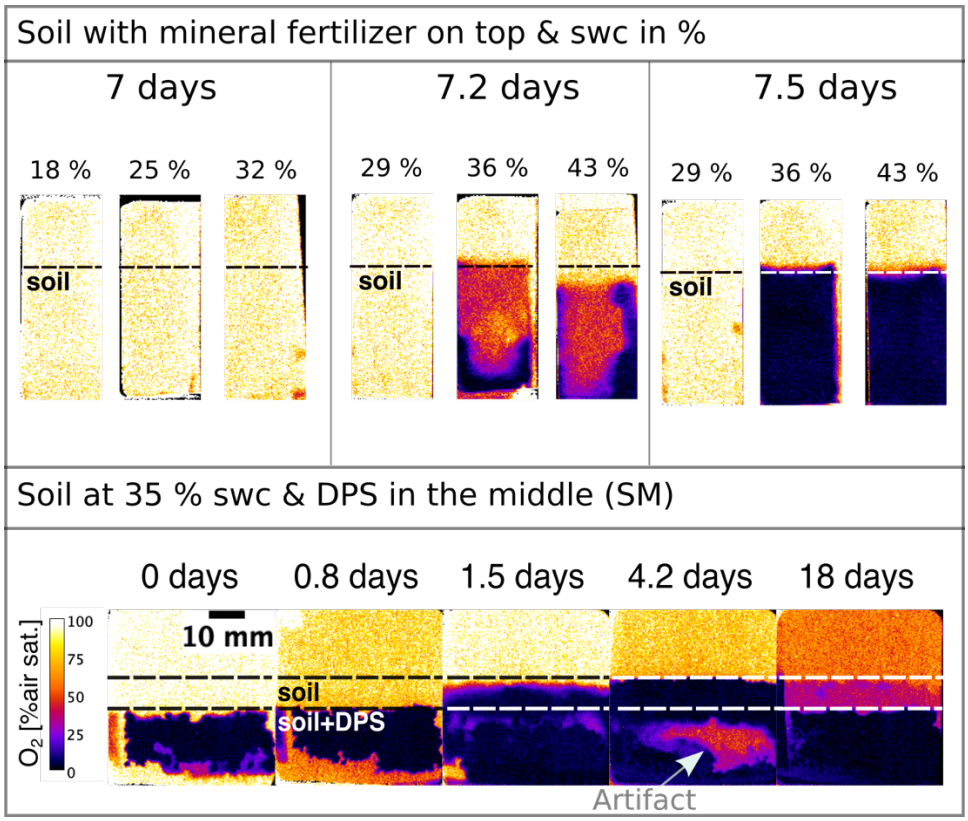

Figure 5. A: False color images of the oxygen (O_2) concentration from Study 5 upon synthetic fertilizer was added and the images were taken for 7 days at the three different soil water contents (swc) (18 %, 25 % and 32 %) showing one of two replicates per soil water content. The image at the timepoint 7.2 days was taken after 11.5 mL of water was added to simulate a rain event. This heightened the soil water contents to 29 %, 36 % and 43 %. Only 8 hours (7.5 days) later the third panel shows the chambers with soil water contents of 36 % and 43 % with anoxic zones. Additionally, the false color images of the O_2 concentration of Study 1 are shown. In this study a soil water content of 35% was maintained and organic fertilizer was applied in the middle part of the packed soil. Imaging was conducted for 18 days.

As soon as the soil's pH value is outside of the dynamic range the false color image in Figure 4A as well as the line profile 1 (LP1) from Soil 1 in Figure 4B show that it is not possible to achieve proper pH measurements. LP1 shows a rather noisy signal due to reaching the detection limit of the pH optode while LP2 and LP3 from Soil 2 result in much better signal qualities as they lie well within the dynamic range. This shows that it is important to consider the optical sensor's working range and to investigate the soil system's characteristics in advance.

Despite the proposed considerations that need to be taken into account before using optical sensors for soil pH analysis we still think it is a good approach for *in-situ* measurements at lower soil water contents. A study by Nielsen *et al.* found that the conventional laboratory pH measurements for soils with suspension samples continuously overestimated the pH compared to *in-situ* pH measurements directly in the field[51]. The *in-situ* pH values were 0.5-0.8 pH units lower than the pH values measured with the extraction method in the laboratory. A similar trend was observed in our studies too. The soils used in the present study were also measured in a soil water suspension (soil:deionized water 1:2.5 w/w) resulting in a soil pH of 5.6 (Soil 1) and a soil pH of 7.7 (Soil 2). The *in-situ* measurements with the pH optodes, however, revealed a soil pH of around pH 5 for Soil 1 and a soil pH of pH 6.6 for Soil 2. This reveals lower pH measurements with the pH optodes here too, with a difference of 0.6 units for Soil 1 and 1.1 units for Soil 2. Possible reasons for the higher pH values measured with the standard laboratory method could be the different modes of operation[47], the release of buffering ions from soil biota due to drying, extracting, and rewetting of soil samples[51] or the change in electrolyte concentration due to dilution of the soil with water. Hence, it should become more common practice to choose *in-situ* pH measurements over the standard method to avoid sample handling artifacts.

Oxygen optodes in non-waterlogged soils. *In-situ* measurements of soil O_2 contents with optical sensors have been investigated in a number of studies, especially in combination with amendments of organic fertilizers[19,23,24]. That is because the availability and spatial distribution of O_2 have immense impacts on C and N cycling as well as greenhouse gas emissions. However, most of these studies were conducted in soils with high soil water contents, partly due to the addition of liquid manure as organic fertilizer. In the present study, we investigated the useability of O_2 optodes related to the difference in O_2 distribution in soils with different gravimetric soil water contents and added a mineral and an organic fertilizer (Figure 5). It can be seen in Figure 5 that oxic conditions dominated for the first seven days after mineral fertilizer was applied to the top of the soil. In contrast, O_2 depletion zones immediately formed when organic fertilizer was applied and mixed within the middle area of the soil, which is due to the more easily biodegradable organic C in the sludge stimulating greater microbial activity. The O_2 depletion zones expanded to the surrounding soil for the next days. On day 4 a small zone of O_2 increase can be seen within the sludge and soil band (Figure 5, bottom). It looks like O_2 production took place. This, however, is very unlikely as there were no plants and therefore plant roots involved in this sample to explain such a rise in O_2 levels. More likely though is the detachment of soil from the optode and O_2 influx from the headspace to that area. This points out how important it is that the sample is in good contact with the optode in order to not misinterpret measured artifacts.

Another challenge that is presented here is the measurement of O_2 dynamics at different soil water contents. This can be seen in Figure 5 in the treatment with mineral fertilizer on top where the soil with gravimetric water contents from 18-32 % is fully oxygenated. And only after increasing the soil water content to 36 % and 43 % an anoxic zone within the soil is formed. This supports the fact that O_2 diffusion becomes limited as soon as the soil becomes more waterlogged. It also shows that air filled pore spaces filled up with water after irrigation, which

supports the increase of the anoxic area. Even though O_2 optodes show a fully oxygenated soil under certain soil water contents it does not necessarily mean that the oxygen concentrations shown with this measurement method depict the whole complexity of the O_2 dynamics. Anoxic zones can be present within soil particles, as shown by Revsbech *et al.*[52] by using a Clark-type microsensor for O_2 within small soil particles. Even though optodes offer a high-resolution measurement together with the possibility of imaging heterogeneities, it also showcases the limitations of this method as it is a bulk measurement compared to even higher resolution methods like microsensors. This together with the need for contact between sample and optode are not necessarily shortcomings of O_2 optodes in soils but rather challenges that need to be considered when designing the study.

Considerations when using optodes in non-waterlogged soils. Overall, the studies show that optodes can be a valuable additional tool in the soil analysis toolbox due to their abilities and the possibility to combine them in the same system if their limitations are taken into consideration. Our results demonstrated the ability to visualize the relative differences in NH_3 concentrations resulting from varying fertilizer amendments and from different soil pH values. On a large scale such changes in concentration could have a big effect. Hence, the advantage of such short-term experiments can be to offer a preliminary assessment of the impact new fertilizers, application techniques and soil-fertilizer interactions could mean on a bigger scale. Therefore, a fast information transfer can be offered due to the reduction of laborious and costly field experiments for soil and fertilizer analysis with high spatiotemporal resolution. The interpretations of the NH_3 optode results, though, still need to be considered as relative values due to a possible humidity dependency and needed calibration improvements to allow more accurate gas phase measurements. Our measurements showed that pH optodes are a great alternative to conventional methods when it comes to measuring pH in soils *in-situ*. Additionally, it is important to be aware of the diminished dynamic range pH optodes operate in compared to potentiometric sensors. In regards to O_2 optodes, we showed that it is not always feasible to measure spatiotemporal dynamics of O_2 if soil water contents are too low. The settings of the biological system need to be considered first regarding soil water content, organic matter, and processes that might occur. Another aspect regarding all optodes is their temperature dependency, which is predictable and can be corrected for[53] or circumvented by keeping the temperature stable throughout laboratory-based experiments. Showcasing the possibilities as well as the challenges of optodes in soil systems could aid to bridge the gap between two fields. This will help to broaden our understanding of complex soil processes and how these are linked to emissions from the agricultural sector by adding missing links of spatiotemporal variations within the soil and at the soil/air interphase.

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