Multimodal Probe Based on ISFET Electrochemical Microsensors for In-Situ Monitoring of Soil Nutrients in Agriculture [†]

Matthieu Joly [1,2], Laurent Mazenq [1], Maurane Marlet [2], Pierre Temple-Boyer [1], Céline Durieu [2] and Jérôme Launay [1,*]

[1] Laboratory for Analysis and Architecture of Systems (LAAS-CNRS), 7 avenue du Colonel Roche, 31400 Toulouse, France; mjoly@laas.fr (M.J.); lmazenq@laas.fr (L.M.); temple@laas.fr (P.T.-B.)

[2] Agronutrition, 3 avenue de l'Orchidée, 31390 Carbonne, France; m.marlet@agro-nutrition.fr (M.M.); c.durieu@agro-nutrition.fr (C.D.)

* Correspondence: jlaunay@laas.fr; Tel.: +33-561-336-6809

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Abstract: We report the design of a silicon chip dedicated to the in-situ monitoring of soil nitrogen cycle in wheat crop. Our study shows that ion-sensitive field effect transistor (ISFET) microsensors are suitable for quick on-site or long-term analysis of nutrients measured directly in soil as opposed to soil extracts analysis. Our pH-ISFET recorded soil pH for six months with results in good accordance with standard sampling method and without any loss of sensitivity. The adaptation of pH-ISFET in pNO_3 and pNH_4-ISFET allowed, for the first time, the in-situ measurements of natural variations of soil nitrogen contents caused by microorganisms' activity

Keywords: ISFET sensor; in-situ soil analysis; multisensor probe; ion-selective membrane; nutrients levels determination; agriculture

1. Introduction

Precision agriculture can help to reduce the environmental impacts of intensive agriculture by determining fertilizers doses over time and at small scale according to soils variability. Thus, our project aims to provide a low-cost, robust and real-time multimodal tool based on ISFET microsensors for in-situ monitoring of soil nutrients.

Since the 2000s, several studies showed the promising future of potentiometric sensors in precision agriculture. Most of them focus on the development of flow injection analysis (FIA) systems in which ion-selective electrodes (ISE) or ISFET are used for nutrients measurements in soil extracts performed on-site or on-the-go [1,2]. Artigas et al. reported the fabrication of pH-ISFET, pK-ISFET, pCa-ISFET and pNO_3-ISFET used directly in soil [3]. Nevertheless, more studies are needed to fully assess some important parameters of ISFET's detection capability in soil: influence of soil moisture, ISFET drift and lifetimes and the in-situ detection of other important nutrients like NH_4^+ ammonium ions.

2. Microsensor Fabrication and Measurement Methods

Our microsystem is fabricated on 4-inch silicon substrates and composed of an ISFET, a miniature solid-state reference electrode, two platinum electrodes for EC measurements and a temperature sensor in MOSFET technology (Figure 1a).

N-channel, normally-off pH-ISFET microsensors were fabricated using a 50 nm silicon nitride Si_3N_4 pH-sensitive layer deposited by Low Pressure Chemical Vapor Deposition (LPCVD) on top of a 50 nm thermal oxide SiO_2 layer. Such ISFET was already studied in previous work, evidencing a quasi-Nernstian sensitivity of around 50 mV/pH [4].
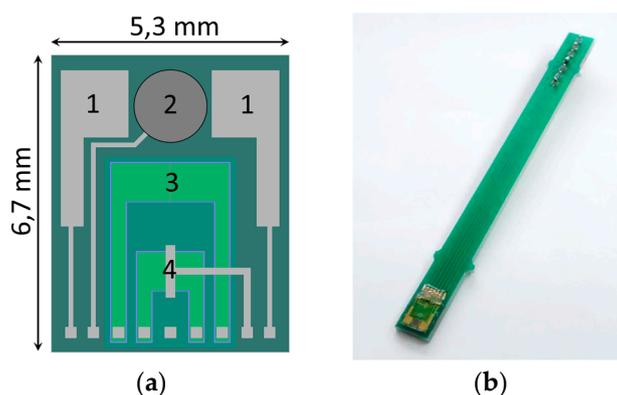
Figure 1. (**a**) Schematic top view of the microsystem. (1) Platinum electrodes for EC measurements; (2) Miniature Ag/AgCl quasi-reference electrode; (3) ISFET micro-sensor; (4) MOSFET for temperature measurement; (**b**) View of the chip after electronic packaging on a printed-circuit board (PCB) and encapsulation.

The detections of ammonium and nitrate ions were allowed by casting 0.5 µL of an ion-sensitive membrane on top of the pH-ISFET Si_3N_4 layer. The membranes were based on fluoropolysiloxane (FPSX) matrix sonicated in tetrahydrofuran with ionophores and ionic additives. It contained: 3.5% (*w/w*) nonactin, 1.5% (*w/w*) KTpClPB, 95.0% (*w/w*) FPSX in the case of pNH$_4$-ISFET; 1.5% (*w/w*) Nitrate ionophore V, 0.9% (*w/w*) TDMACl, 97.6% (w/w) FPSX in the case of pNO$_3$-ISFET. The FPSX was chosen for its good adhesion properties on Si_3N_4 surface leading to an improved sensor's lifetime.

The performances of the ISFETs were examined using a specific potentiometric ISFET-meter electronic interface with drain voltage V_{ds} and current I_d fixed to 2.0 V and 0.1 mA respectively. A silver silver chloride double junction electrode was used as a reference electrode in aqueous solution and in soils. Sensitivity tests were performed by increasing the activity of the primary ions using a 0.1 M ammonium nitrate NH_4NO_3 aqueous solution. Selectivity coefficients were obtained with the Fixed Interference Method (FIM). Measurements in soils were done by inserting the ISFET's PCB directly into the soil in a way that the sensitive part of the sensor was in close contact with the soil. All measurements were carried out at room temperature.

3. Results and Discussion

3.1. In-Situ Detection of Soil pH

We first studied the detection capability of pH-ISFET microsensors inserted directly into the soil in order to prove the feasibility of a direct measurement method as opposed to soil extract analysis.

Two silty-clay soils were first analyzed according to the standard method where a glass pH electrode is used in a 20 vol % suspension of soil in water. It led to pH values of 4.7 and 8.4. After a three points-calibration in pH buffer solutions (pH 4, 7 and 10), quick in-situ measurements of pH-ISFET were recorded for both soils at different soil moisture levels (Figure 2a). The results are in great accordance with the standard method. Indeed, the maximum error was only 0.5 pH unit and there was no correlation with soil moisture. In-situ measurements were even possible at soil relative water content of 40% which is below typical wheat growths conditions. With its small measurement errors relative to an agriculture application and its robustness, pH-ISFET is fully compatible for quick on-field pH determination.
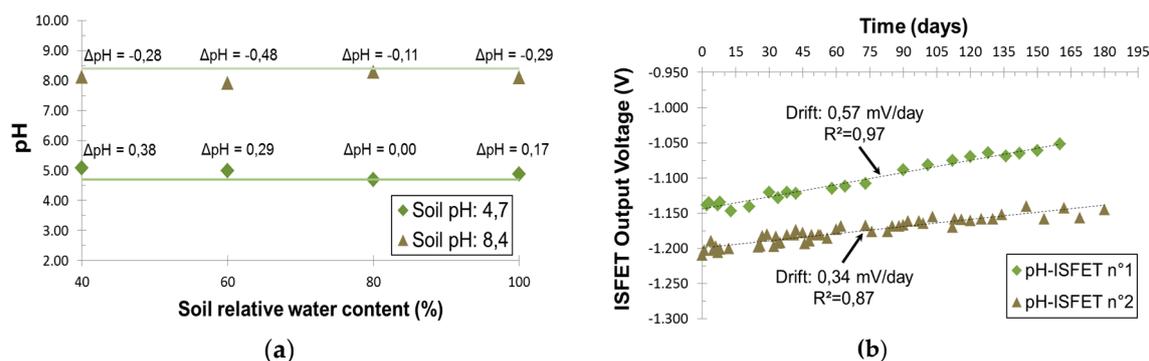
Figure 2. (**a**) Comparison of pH measurements performed by pH-ISFET at different soil moisture levels in an acid and a basic silty-clay soil. The difference with the standard method of soil pH determination (green lines) is indicated above each measurement point; (**b**) Time evolution of the output voltage of two pH-ISFET inserted in a silty-clay soil for six months.

Furthermore, we recorded for six months the signal drift of two pH-ISFET directly inserted in pots of silty-clay soil using the simple packaging showed on Figure 2b. This study aimed at assessing the pH-ISFET lifetime in soil to know if a monitoring during all wheat growth stages could be possible. Relative water content (75%) was kept constant thanks to a daily subirrigation. Both microsensors displayed a linear ($R^2 \geq 0.87$) and relatively small drift (<0.6 mV/day). The ageing of pH-ISFET caused by soil environment was evaluated by a comparison of their sensitivity before and after the experiment. Both ISFET kept their initial sensitivity (51 mV/pH). Thus, our pH-ISFET showed a lifetime in soil of at least six months making the project of a long-term monitoring of soil nutrients possible.

3.2. In-Vitro Detection of Nitrate and Ammonium Ions

Calibration curves obtained for pNO_3- and pNH_4-ISFET are shown in Figure 3a. Both sensors exhibited a linear response to the concentration of primary ions with a quasi-Nernstian sensitivity of 59.0 mV/pNH_4 and 56.2 mV/pNO_3, respectively. The limits of detection (LOD) calculated from the intersection of the two extrapolated segments of the calibration curve, were 3.2 μM and 17 μM, respectively. Both the LOD and the linear range are in accordance with standard values of mineral nitrogen in field conditions. Thus, our probe should meet the requirements for a soil study application.
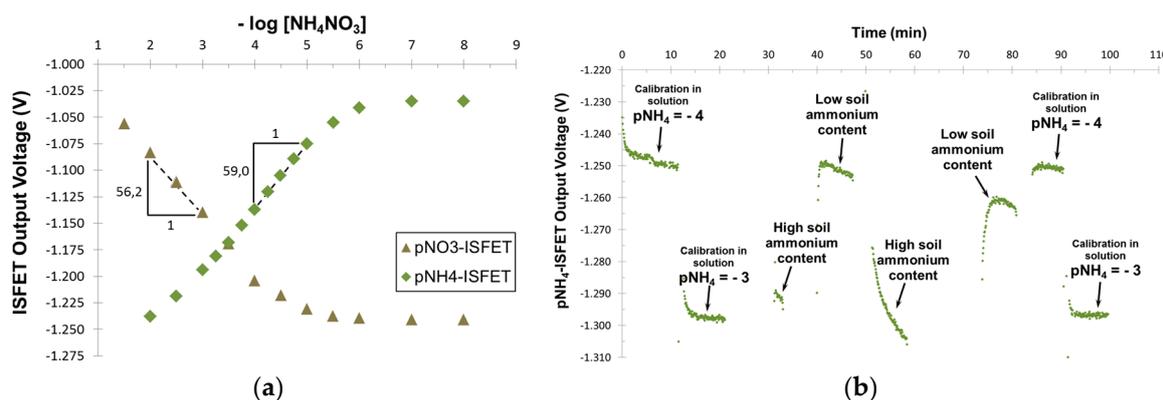

Figure 3. (**a**) Calibration curves of the pNO_3-ISFET and pNH_4-ISFET in aqueous solution of NH_4NO_3; (**b**) Plot vs. Time of pNH_4-ISFET signal. The measurements are first done in two calibration solutions, and then in high and low nitrogen content soils before being checked again in the two same calibration solutions.

A specific care was taken for the determination of FIM selectivity coefficient (Table 1) as direct measurements in soils could obviously not be done without the presence of interfering ions.

Table 1. Selectivity coefficients (log K_{ij}) obtained with the Fixed Interference Method for pNH4- and pNO3-ISFET. The constant concentration of interfering ion is indicated in brackets.

Interfering Ion	Log K_{NH4^+,C^+}	Interfering Ion	Log K_{NO3^-,A^-}
K^+ (0.005 M)	−1.2	Cl^- (0.005 M)	−1.6
Na^+ (0.1 M)	−3.0	$H_2PO_4^-$ (0.05 M)	−2.9
Ca^{2+} (0.05 M)	−4.8	PO_4^{3-} (0.1 M)	−4.0
Mg^{2+} (0.1 M)	−5.0	SO_4^{2-} (0.1 M)	−3.1
Li^+ (0.01 M)	−4.1	CH_3COO^- (0.01 M)	−2.5

Overall, both functionalized ISFET exhibited good selectivity coefficients towards the main ions likely to be found in soil. As expected with nonactin ionophore, potassium K^+ ion interfered the strongest with our pNH4-ISFET. Despite the use of Nitrate Ionophore V, the relative responses of our pNO3-ISFET towards interfering ions indicate a Hofmeister behavior with a poor selectivity towards chloride. Nevertheless, with exception to coastal soils in which high content in chloride is expected, our pNO3-ISFET should be selective enough for an application to soil monitoring.

3.3. In-Situ Detection of Ammonium Ions

Finally, we tested our pNH4-ISFET in silty-clay soils of varying nitrogen content. Starting with two pots of the same soil, we obtained two distinct nitrogen content soils by putting them respectively at 38 °C and 4 °C for one month. Indeed, nitrogen mineralization by soil microorganisms is a temperature-dependent process. Measurements performed into these soils directly after calibration allowed to discriminate the two levels of nitrogen. We successfully recorded an increase in ammonium concentration caused by the stimulation of nitrogen mineralization by soil microorganisms (Figure 3b).

4. Conclusions

Our pH-ISFET succeeded in measuring acidic and basic soils, for short and long periods of time, with only an elementary packaging and in avoiding the need for a time-consuming step of soil sampling. These results validated the feasibility of a direct measurement method in soil. Then, our nitrogen-functionalized ISFET exhibited quasi-Nernstian sensitivity and detection limit in accordance with standard values in field conditions. Our pNH4-ISFET was able to differentiate two soils of different nitrogen content. Overall, this work highlights the promising future of the ISFET technology for precision agriculture.

Conflicts of Interest: The authors declare no conflict of interest.

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