Precision Agric (2017) 18:152–168
DOI 10.1007/s11119-016-9452-y

A mobile lab-on-a-chip device for on-site soil nutrient analysis

M. Smolka[1] · D. Puchberger-Enengl[1,2] · M. Bipoun[2] · A. Klasa[3] · M. Kiczkajlo[3] · W. Śmiechowski[3] · P. Sowiński[4] · C. Krutzler[5] · F. Keplinger[1] · M. J. Vellekoop[2]

Published online: 24 May 2016

Abstract In this paper, a mobile sensor for on-site analysis of soil sample extracts is presented. As a versatile tool for scanning ion concentrations in liquid samples, it especially allows the analysis of NO_3, NH_4, K and PO_4. The sensor mainly consists of a microfluidic chip in which the sample ions are separated in an electric field (capillary electrophoresis) and the individual ion concentrations are detected by a conductivity measurement. For the adaption of the device to field conditions, two major concerns were addressed. Firstly, nano-porous material was used as a barrier between the sample container and the analysis channel of the microfluidic chip. This prevents pressure driven leakage of the sample into the chip due to non-horizontal orientation of the device. Secondly, a new method for the injection of the sample into the chip was used. It reduces the number of fluidic connections between chip and operation device to three instead of the commonly used four connections. The sensor performance was tested on multi-ion solutions with calibration series for NO_3, NH_4, K and PO_4. For the first on-site test, a quick soil nutrient extraction procedure with water was used. The sensor data was compared to standard laboratory results. The potential of the sensor for soil nutrient analysis is

✉ M. J. Vellekoop
mvellekoop@imsas.uni-bremen.de

F. Keplinger
franz.keplinger@tuwien.ac.at

[1] Institute of Sensor and Actuator Systems (ISAS), Vienna University of Technology, Gusshausstraße 27-29, 1040 Vienna, Austria

[2] Institute for Microsensors, -Actuators and -Systems (IMSAS), MCB, University Bremen, Otto-Hahn-Allee 1, 28359 Bremen, Germany

[3] Department of Agricultural Chemistry and Environmental Protection, Warmia and Mazury University in Olsztyn, ul. Oczapowskiego 8, 10-744 Olsztyn, Poland

[4] Department of Soil Science and Land Reclamation, Warmia and Mazury University in Olsztyn, Plac Łódzki 3, 10-727 Olsztyn, Poland

[5] Austrian Center for Medical Innovation and Technology GmbH (ACMIT), Viktor-Kaplan-Straße 2/1, 2700 Wiener Neustadt, Austria

discussed together with required improvements of the sensor performance and of the nutrient extraction procedure.

Keywords On-site soil nutrient sensor · NPK · Microfluidics · Lab-on-a-chip · Capillary electrophoresis · Capacitively coupled conductivity detection C^4D · Capacitively coupled conductivity measurement C^4M

Introduction

State of the art of soil nutrient analysis

Monitoring soil nutrients is an essential task for sustainable agriculture (Bindraban et al. 2000; Roberts et al. 2013). In this respect, it is important to know if the soil gets back what has been taken from it during crop growth and if the procedures for soil fertilisation do not exceed the plant's demand. With this information, environmental hazards may be reduced and expenses optimised.

The availability of soil macronutrients N, P and K in the ionic form of NO_3, NH_4, PO_4 and K is of special interest, as these ions are directly available for plant roots. Amongst them, NO_3 is of central importance for plant physiology (see Ho and Tsay 2010). In standard laboratory tests, these ions are extracted by shaking soil samples with an extraction solution and the ion concentrations in the extracts are typically measured with Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) or Atomic Absorption Spectroscopy (AAS) after a filtration procedure. The cost of this analysis is high, the sample treatment is complicated (e.g. soil samples have to be stored at low temperatures (4 °C) for N concentration analysis) and results are obtained only after a delay, especially in regions with low density of laboratory infrastructure. Devices for fast on-site analysis of soil nutrients are therefore under intensive research.

Many researchers have tried simplified extraction procedures, which allow on-site measurements in soil sample extracts and subsequent measurement with portable devices. Ion selective sensors [ion selective electrodes (ISE) or ion selective field effect transistors (ISFET)] were used by Artigas et al. (2001), Kim et al. (2007) and Shaw et al. (2013). Colorimetric test strips with optical read out devices have been frequently investigated (Scholefield and Titchen 1995; Wetselaar et al. 1998). These techniques have to be tuned to the analyte ions by the selection of suitable ion selective membranes or test strips. Hence the simultaneous measurement of various nutrients is complicated or demands the use of several instruments.

Fertility related parameters of soil samples have been investigated with reflectance measurements on soil samples in the visible, near and mid-infrared ranges of the electromagnetic spectrum [soil reflectance spectroscopy (SRS), for literature overviews see e.g. Schirrmann et al. (2013), An et al. (2014), Vohland et al. (2014)]. Together with site-specific multivariate calibrations (see e.g. Shi et al. (2012) and Viscarra Rossel and Behrens (2010) and cited papers therein) remarkable results were achieved e.g. in measurements of soil organic matter (SOM) and total nitrogen content (N_{tot}). The short term availability of N, P and K was studied with SRS as well but especially for NO_3 concentrations, a key parameter for short term nutrient availability, no clear correlation with

standard tests was found [$R^2 = 0.5$ by Shibusawa (2003) and $R^2 = 0.0$ by Viscarra Rossel et al.(2006)].

Several mobile units for testing of soil fertility parameters from a field vehicle have been investigated (on-the-go soil sensor systems). Soil samplers on a tractor allowing on-the-go NO_3 measurements with ISE or ISFET sensors were presented by Birrell and Hummel (1997, 2001), Sibley et al. (2010). Vehicles using SRS can directly scan soil parameters without the need for a sampling mechanism. A system presented by Christy et al. (2006) was marketed by Veris Technologies, USA. The technique showed promising performance in measurements of fertility related parameters like SOM and N_{tot} (Kodaira and Shibusawa 2013; Schirrmann et al. 2013). Kodaira and Shibusawa (2013) evaluated the correlation of NO_3 concentrations predicted by SRS with laboratory measurements but found a low correlation with $R^2 = 0.5$.

In summary, a technology for sensing ionic forms of soil macronutrients N, P and K with one portable device is still missing. The present study expands the techniques of mobile ion concentration measurements in soil sample extracts with recent research in the fields of Microfluidics. This research area aims at the integration of laboratory procedures with miniaturised systems (lab-on-a-chip (LOC) systems or micro total analysis systems (μTAS)) with functionalities like fluid handling (mixing, separation, pre-concentration), activation of micro-reactions, chemical analysis and many more. The central interest lies in miniaturised portable and user-friendly devices for environmental analysis, chemistry, medical technology and many more applications.

Objectives

A LOC device capable of measuring the most important plant nutrient ions in soil sample extracts is presented. The paper focuses on the following objectives:

1. Demonstration of the sensitivity of the LOC device to macronutrient ions NO_3, NH_4, K and PO_4.
2. Characterization in field conditions with water-extracted soil samples including comparisons to standard laboratory measurements.
3. Discussion about the potential of the sensor for measurements of additional ions (Cl, SO_4, Ca and Na).
4. Discussion of needed advancements of the sensor performance as well as of the soil nutrient extraction procedures.

Materials and methods

Basics of capillary electrophoresis

The sensor presented uses the principle of capillary electrophoresis (CE) (Ewing et al. 1989; Kuhr 1990; Roth et al. 1991) to separate different ion species of a liquid sample by an electric field and to move them past a detector one after another. CE is performed in a buffer-filled capillary to which the sample is introduced from one end, so that a few per cent of the capillary length are filled. With an electric voltage between the ends of the capillary, the sample ions migrate with a speed dependant on their size and charge. This causes the separation of the ion species into plugs which independently pass an ion detector at the opposite capillary end. Each ion plug causes a peak in the recording of the detector

signal (electropherogram) and the peak area scales linearly with the ion concentration (at low concentrations where the ion activity equals the ion concentration).

CE was originally setup with tubular capillaries with typical length of several hundred mm. The long capillaries allow good separation quality in numerous analytical applications. In cases where also short capillaries (length of a few tens of mm) yield separation of all analytes (e.g. analysis of small inorganic ions), the integration of the setup to a microfluidic chip is an attractive alternative [microchip capillary electrophoresis MCE, see e.g. Fercher et al. (2010a), Laugere et al. (2003), Tanyanyiwa and Hauser (2002)]. This allows strongly miniaturized setups especially for mobile devices [see e.g. Becker et al. (2008), Gärtner et al. (2009), Kubán et al. (2007), Puchberger-Enengl et al. (2013), Smolka et al. (2013)].

Setups of CE for the analysis of soil nutrients have been presented by Howald et al. (1995) and O'Flaherty et al. (2000). Due to the set-up with classic capillaries, the devices are bulky and not suitable for untrained users. Studies of soil nutrient measurements based on MCE devices have not been presented so far.

CE and MCE ion detectors are often based on a conductivity measurement [for general reviews see e.g. Guijt-van Duijn et al. (2004), Swinney and Bornhop (2000), Vandaveer et al. (2004)]. As the conductivity is influenced by all charged sample components, this principle is practically universal for ion detection (Coltro et al. 2012). A frequently used conductivity measurement is built with two electrodes which are separated from the liquid in the analysis capillary by an insulating material (the capillary wall in the case of CE or an insulation layer in MCE). One electrode is excited with a high frequency (HF) voltage signal. The second electrode is connected over a sensitive current measurement device to the ground potential of the HF source. This setup generates and measures a local capacitively coupled current in the liquid of the capillary. The measured signal varies when passing ions change the conductivity near the electrodes. This technique has been studied by many researchers on microchips and is typically called capacitively coupled contactless conductivity detection or measurement (C^4D or C^4M) (Fercher et al. 2010b; Fracassi da Silva and do Lago 1998; Kubáň and Hauser 2009; Zemann et al. 1998).

The research on the device presented was focused on the set-up of a stable operating mobile device. In practice, such a mobile device will not be kept aligned horizontal and thus pressure differences will occur between the liquid containers used. This effect complicates the liquid analysis, which demands well-defined injection of nanoliters of the sample (Karlinsey 2012). Stable conditions of the liquids in the measurement system have been achieved by the integration of a nano-porous filter at the sample inlet of the chip,

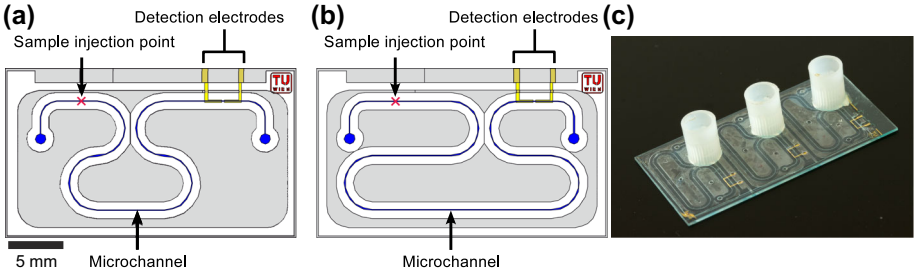

Fig. 1 Two chip layouts have been developed, one with a 44 mm long channel (**a**), one with a 64 mm long channel (**b**). The design (**a**) was used for all measurements in this paper. Unit of three fully processed chips with mounted sample reservoirs (**c**)

which drastically dampens pressure driven liquid flow but allows penetration of ions driven by electric fields. Furthermore, the presented device is simpler than other designs as only three microfluidic connects are used and no mechanical pumping of the sample is required.

Device fabrication

The central part of the nutrient sensor is the MCE chip, which mainly consists of a microchannel with the C^4D detection electrodes at one end. The channel has one inlet to inject a buffer solution and one inlet for the sample injection (sample injection point). Designs of the microchip and prototype chips are shown in Fig. 1. Each chip is 16 mm × 26 mm. In all measurements in this paper, the channel was 44 mm long (distance between sample injection point and detection electrodes), 100 µm wide and 55 µm high.

The chips were fabricated on a rectangular glass wafer (100 mm × 100 mm). In total, 18 chips were realized per wafer. The fabrication steps are shown in Fig. 2. Briefly, the measurement electrodes are structured on the glass substrate by a standard lift-off process. After application of a 17 µm dry film photoresist (Ordyl, Elga Europe, Italy) as an insulation layer, the channel is patterned in another 55 µm thick resist layer by

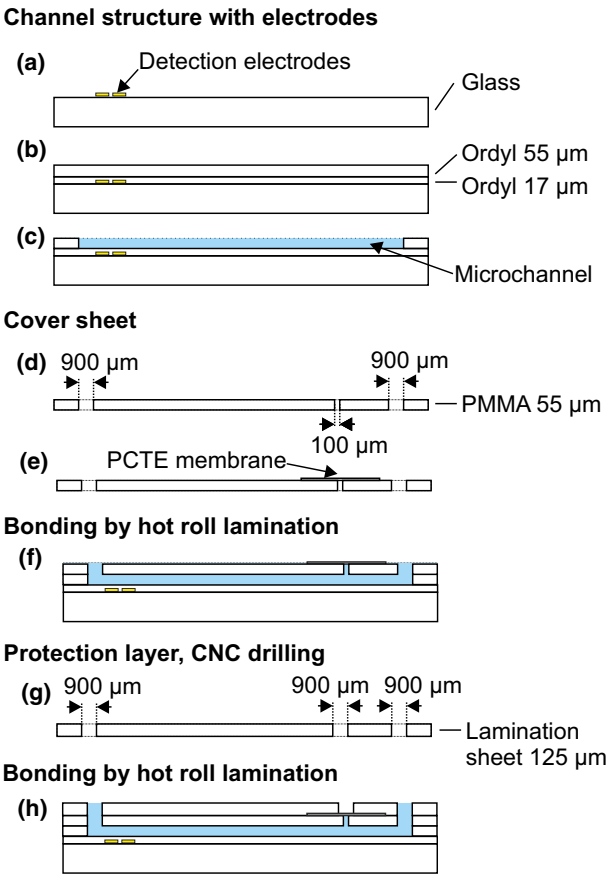

Fig. 2 The chip production process in cross-section along the channel: **a** Forming of the Cr–Au electrodes by evaporation and lift off process. **b** Hot roll lamination of 17 µm Ordyl dry film resist, flood exposure with ultraviolet light (UV) and deposition of 55 µm Ordyl dry film resist. **c** Generation of the channel structure by selective UV exposure and development of the photosensitive dry film resist Ordyl. **d** Processing of the polymethylmethacrylate (PMMA) cover sheet by computerized numerical control (CNC) drilling. **e** Bonding of a nano-porous polycarbonate track etched (PCTE) membrane. **f** Bonding of the cover sheet to the chip by hot roll lamination. **g** Processing of the protection layer by CNC drilling. **h** Bonding of the protection layer by hot roll lamination

photolithography. The channel is sealed by a cover sheet containing a nano-porous membrane. Further details are given in Smolka et al. (2013).

Measurement set-up and injection procedure

Figure 3 depicts the schematic of the chip in the measurement setup. First, 0.16 ml of the sample is inserted into the sample reservoir with a manual pipette. The buffer inlets of the chip are connected to tubes, each leading into a bottle. One of these bottles (pressure bottle) can be switched to 100 kPa over-pressure to pump the buffer through the chip into the second bottle (collection bottle). The over-pressure is generated with an air-filled syringe of a syringe pump (neMESYS, cetoni GmbH, Germany). This procedure is used to fill the chip with buffer and to flush it after each measurement.

A Pt electrode dips in each bottle and into the sample reservoir. By applying electric voltages between the electrodes, the electrophoretic effect transports the sample ions into the chip and through the micro-channel.

Without electric voltage, the nano-porous membrane blocks the sample flow into the micro-channel. This prevents self-driven injection by pressure differences due to unequal levels of the liquid in the reservoirs or due to diffusion. In contrast, a high injection voltage (U_{inj}) between sample reservoir and the pressure bottle causes an electrophoretic motion of sample ions through the pores of the membrane into the micro-channel. After switching off of the injection voltage, a sample plug has entered the chip. The duration t_{inj} of U_{inj} defines the length of the sample plug in the microfluidic channel. This process is visualised in Fig. 3 with a voltage U_{inj} (typically -2000 V) between sample reservoir and pressure bottle.

Varying the plug length allows adjusting the sensor properties: a prolonged injection time increases the amount of injected sample and therefore increases the sensitivity of the sensor. However, excessive sample loading leads to overlapping peaks. Thus the injection time has to be suited to the investigated chemical compounds.

After sample injection, the high voltage U_{sep} between the two bottles is applied. To suppress further flow of sample ions into the micro-channel, the voltage in the sample reservoir is adjusted to get a slight current flowing back to the sample electrode. This means that all remaining sample ions are drawn back towards the sample electrode, as is

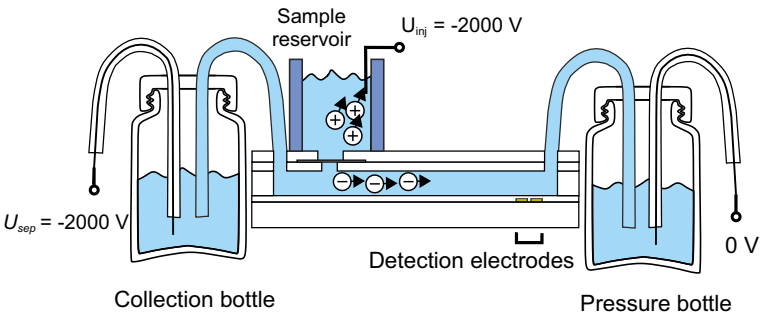

Fig. 3 Schematic of the sample injection: a high potential difference U_{inj} is applied between pressure bottle and sample reservoir. This causes sample ions to pass the membrane and to enter the separation channel. In the case depicted, the voltage pattern is suited for anion injection

the case for a voltage of −1600 V in the sample reservoir and −2000 V in the collection bottle.

The applied voltage pattern causes the injected ion plug to migrate towards the detection electrodes. During the motion, the individual ion species split according to their electrophoretic mobilities and ion packages pass the detector independently. The detector signal is recorded (electropherogram) and the peak areas are evaluated with an algorithm for baseline and peak search and peak area integration (implemented in OriginLab, OriginLab Corporation, USA).

The voltage patterns described are suited for the injection and separation of anions. For cation measurements, all voltages have to be reversed. A summary of the applied voltages and switching times is given in Table 1.

Reagents and samples

Chemicals were purchased from Sigma-Aldrich GmbH, Austria. All chemical solutions were prepared with de-ionised water from a Millipore system (Hydrolab, Poland, ion exchange resin H^+/OH^-, electric conductivity ≤0.2 µS/cm). The buffer consisted of 30 mmol/l DL-histidine (His), 30 mmol/l 2-(*N*-morpholino)ethanesulfonic acid (Mes), 4 mmol/l crown-6-ether and 0.1 % methyl cellulose at pH of 6.5.

All calibration samples were prepared in 30 mmol/l His/Mes solution from 200 mmol/l stock solutions of KNO_3 $(NH_4)H_2PO_4$, $CaCl_2$, and Na_2SO_4. All filtered soil extracts were mixed with 200 mmol/l His/Mes stock solution in 5.55–1 volume ratio, so that the same concentration of 30 mmol/l His/Mes was reached. This high background concentration of 30 mmol/l His/Mes increases the sample conductivities so that the effect of varying sample composition on the injected sample amount is reduced [effect of field amplified sample stacking, see e.g. Bharadwaj and Santiago (2005)]. Further on, the addition of His/Mes buffer stabilises the sample pH around 6.5 where PO_4 is mainly present in the single charged form $H_2PO_4^-$. In the following discussions of electrophoresis experiments, PO_4 and $H_2PO_4^-$ are therefore used synonymously.

Table 1 Steps of the measurement procedure for anion and cation analysis (U_{inj} and U_{sep} are defined in Fig. 3)

Step		U_{inj} (V)	U_{sep} (V)	Duration (s)
Anion measurement				
1	Flush 15 s, wait 15 s			30
2	Injection	−2000	−2000	0.6
3	Separation	−1600	−2000	60
4	Flush 15 s, wait 15 s			30
5	Injection	−2000	−2000	1.2
6	Separation	−1600	−2000	100
Cation measurement				
7	Flush 15 s, wait 15 s			30
8	Injection	+2000	+2000	0.6
9	Separation	+1600	+2000	60

Flushing starts directly after each measurement, enabling a continuous measurement sequence

On-site soil nutrient extraction and reference measurements

The sensor was tested with a soil nutrient extraction with de-ionised water (DIW), the concentrations of the four macro-nutrient ions in the DIW extracts are named C_{NO_3}, C_{NH_4}, C_K, C_{PO_4}. DIW extraction was chosen for this first sensor test, as no additional ions are added to the extracted sample. Cross-sensitivities to high background ion concentrations are thus avoided. This procedure is expected to be adequate for on-site extraction of NO_3^- as good correlations to KCl extraction were shown in a measurement series by Shaw et al. (2013). In contrast, the extracted amount of the macronutrients NH_4^+, K^+ and PO_4 does typically not correlate to extracted amount with standard solutions like $CaCl_2$ for NH_4^+, Ca acetate lactate (CAL) for K^+ and PO_4. DIW extraction is therefore yielding the water-extractable amount of these three ions.

14 soil samples were taken from experimental fields of Warmia and Mazury University in Olsztyn, Poland (53°46′N, 20°28′E). The samples represented four different soil types: Haplic Cambisol, Stagnic Luvisol, Gleyic Phaeozem and Hortic Anthrosol. At each sampling point, three partial soil samples were taken using an Edelman Auger from the topsoil down to a depth of 300 mm. These partial samples were mixed to obtain 200 g of fresh mass. The mixed samples were ground with pestle and mortar and sieved through a 1 mm mesh to discard organic debris and large particles. For the on-site extraction, 15 g of the sieved soil were dosed with a portable scale.

The remaining sieved soil was frozen and sent to a soil laboratory for extraction and analysis. The values of C_{NH_4} and C_{PO_4} in the samples obtained were too low to be quantified by the sensor (see results in Sect. 3). Thus the laboratory procedure is only described for NO_3^- and K^+. Other ion concentrations like SO_4^{2-}, Ca^{2+} and Na^+ were not quantitatively considered in this first sensor test.

The following laboratory extraction and analysis methods were used:

- NO_3^- was extracted in un-buffered 1 % solution of K_2SO_4 and the ion concentrations were measured by colorimetry.
- K^+ was extracted with Egner–Riehm's method [see e.g. Oreshkin (1980)] in 0.1 mol/l calcium lactate (buffered at pH 3.6) and the ion concentrations were measured by atomic emission spectroscopy (AES).

For the on-site extraction, each 15 g soil sample was mixed with 30 ml of de-ionized water in a 50 ml plastic container (1:2 ratio of soil to water) and shaken for 15 min with an orbital mini shaker (rotation frequency 1 Hz). The suspension was filtered through 0.22 µm syringe filters (combined glass and polymer filter from Carl Roth GmbH, Austria). 11 ml of filtered extract was produced for each sample. 1 ml was taken for the sensor measurements. 0.18 ml of concentrated His/Mes buffer (200 mmol/l) was added to adjust the concentration of 30 mmol/l His/Mes buffer in the sample. The remaining 10 ml of the filtered soil sample extract were frozen and sent to a chemistry laboratory for measurements of NO_3^-, NH_4^+, K^+ and PO_4 ion concentrations by inductively coupled plasma atomic emission spectroscopy.

Results and discussion

Measurement parameters

Solutions containing Cl^-, NO_3^-, SO_4^{2-}, $H_2PO_4^-$, NH_4^+, Na^+, Ca^{2+}, and K^+ were used for the sensor calibration. The concentrations of NO_3^-, NH_4^+, $H_2PO_4^-$ and K^+ were

varied in the range of 5–500 µmol/l. The other ions were added for testing the complete separation of the typical soil sample matrix. The maximum injection time that allowed separation of the investigated ions was 0.6 s. The sensitivity of the $H_2PO_4^-$ measurement was only half the values for NO_3^-, K^+ and NH_4^+. This can be explained by the comparably low electrophoretic mobility of $H_2PO_4^-$ ions, which causes lower signal response of the detector.

The sensitivity of the $H_2PO_4^-$ measurement can be increased with a longer injection time. With t_{inj} of 1.2 s, it reaches a similar value to NO_3^-, K^+ and NH_4^+ measurements with 0.6 s injection time. However, the NO_3^- peak is covered by the Cl^- and SO_4^{2-} peaks and can no longer be resolved (see Fig. 4). Thus this sensitive $H_2PO_4^-$ measurement has to be done in a separate run.

Table 1 shows all steps of a nutrient measurement including the optional measurement of $H_2PO_4^-$ with increased sensitivity. Prior to each measurement, the chip is flushed with fresh buffer by applying 100 kPa over-pressure in the pressure bottle for 15 s. Pressure differences are equilibrated during a delay time of 15 s before the measurement begins with the injection of sample anions. The flushing procedure is automatically started again after each ion separation. Each measurement is repeated three times for statistical evaluations.

Sensor calibration

Each measurement day started with sensor calibration using the standard solutions. The standard concentrations of NO_3^-, NH_4^+, K^+ and $H_2PO_4^-$ were 5, 10, 50, 100, 200, 500 µmol/l ± 5 %. Several calibration solutions were measured again throughout the day to characterize the reproducibility of the calibration measurements. All these calibration measurements were considered in the subsequent evaluations (see Fig. 5). A new chip was used each day to avoid contamination by bio fouling.

The peak areas in each electropherogram were calculated and basic sensor parameters were evaluated for each analyte ion (results in Table 2):

- *Calibration constant* slope of the trend line in the graph of ion concentration versus peak area.
- *Average noise level* standard deviation of the detector signal from the baseline in the time before the peak arrival (10–25 s).
- *Limit of detection (LOD)* ion concentration which generates a peak three times higher than the average noise level; lowest concentration which can be detected.

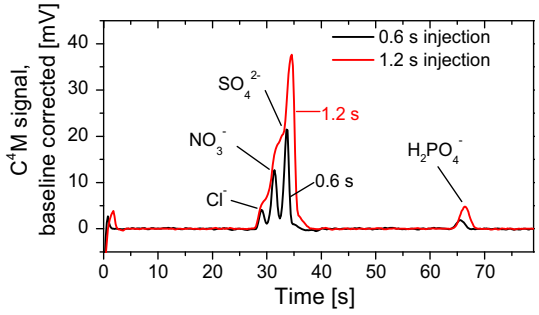

Fig. 4 Electropherograms of 50 µmol/l Cl^-, 100 µmol/l NO_3^-, 100 µmol/l SO_4^{2-} and 50 µmol/l $H_2PO_4^-$ with two different injection times

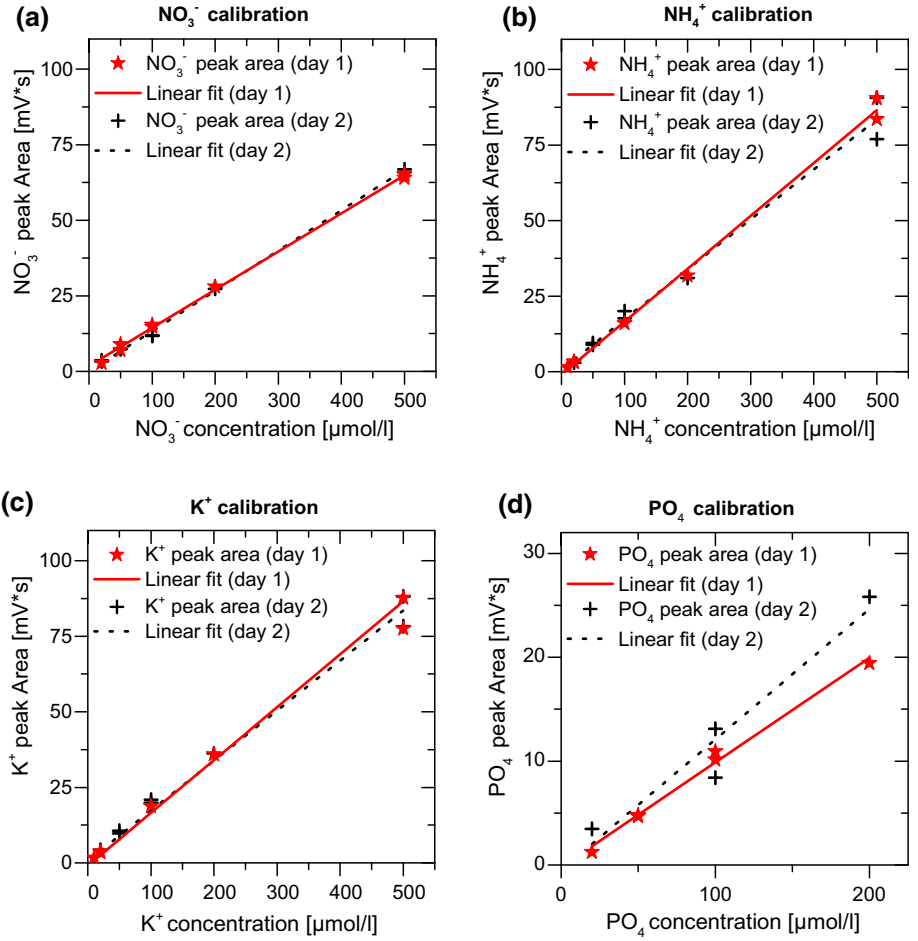

Fig. 5 Calibration curves for the macro-nutrients NO_3^- (**a**), NH_4^+ (**b**), K^+ (**c**), $H_2PO_4^-$ (**d**) measured on two different days

Table 2 Parameters from the calibration measurements of Fig. 5: calibration constant, coefficient of determination (R^2), limit of detection (LOD), limit of quantification (LOQ)

	Calibration constant (µmol/l/mVs)		R^2		LOD (µM)	LOQ (µM)
	Day 1, chip 1	Day 2, chip 2	Day 1, chip 1	Day 2, chip 2		
NO_3^-	7.9	8.3	0.999	0.997	3	10
NH_4^+	6.2	6.1	0.983	0.996	2	7
K^+	6.0	5.7	0.991	0.990	3	10
$H_2PO_4^-$	8.0	9.9	0.989	0.902	10	33

– *Limit of quantification (LOQ)* ion concentration which generates a peak ten times higher than the average noise level; lowest concentration which can be quantitatively evaluated.

The calibration curves in Fig. 5 show the linear response of the sensor to all four macro-nutrient concentrations. The calibration curves of $H_2PO_4^-$ show stronger variation than the curves of the other three ions. This might be caused by the lower and broader peaks of $H_2PO_4^-$ which increase the influence of noise on the peak area evaluation, but more tests are necessary for statistical statements.

Soil nutrient analysis

Typical measurements of DIW soil sample extracts are presented in Fig. 6. The anion signal contains peaks of NO_3^- and SO_4^{2-} (Cl^- concentration was below LOD in the chosen example). $H_2PO_4^-$ values were below LOQ in all 14 soil samples, thus no peak signals are shown here. The corresponding cation curve contains peaks of NH_4^+, K^+, Ca^{2+} and Na^+ (further peaks were not identified). The ion concentrations of NO_3^-, NH_4^+ and K^+ can be calculated by multiplying the peak area values with the calibration constants of Sect. 3.2. SO_4^{2-}, Ca^{2+} and Na^+ concentrations were not evaluated.

The macro-nutrient measurements yielded the following result.

NO_3^- measurement

All sensor values of C_{NO_3} were above LOQ so that a comparison to the laboratory references can be done.

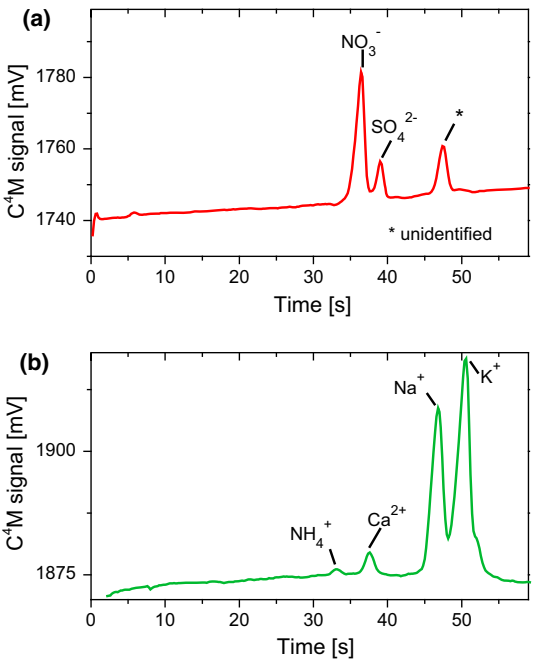

Fig. 6 Typical electropherograms for anion (**a**) and cation (**b**) measurement (before baseline correction)

Sensor versus laboratory measurements of on-site soil sample extracts: Sensor and laboratory values of C_{NO_3} measured in the on-site soil sample extracts are compared in Fig. 7a. The strong correlation with $R^2 = 0.985$ demonstrates the potential of the sensor for on-site NO_3^- measurements. This result resembles the findings obtained with an ISE [$R^2 = 0.968$–0.989 by Shaw et al. (2013), $R^2 = 0.98$ by Kim et al. (2007)], an ISFET [$R^2 > 0.9$ by Birrell and Hummel (1997)] and colour reaction strip sensors [$R^2 = 0.96$–0.97 by Wetselaar et al. (1998)].

On-site versus laboratory extraction: The sensor data of the on-site soil sample extracts was compared to laboratory data after the standard soil extraction procedure in Fig. 7b. The strong correlation with $R^2 = 0.950$ confirms that the on-site soil nutrient extraction is an alternative to the standard laboratory extraction procedure in agreement with Shaw et al. (2013).

NH_4^+ measurement

The C_{NH_4} values were below LOQ except in two cases so that no comparison between laboratory and sensor measurements can be done. Anyhow, the average percentage of C_{NH_4} of the mineral nitrogen concentration (sum of C_{NO_3} and C_{NH_4}) was only 6.4 %. NH_4^+ was therefore a minor source of plant available N. For analysis of the sensor performance in C_{NH_4} measurements, NH_4^+ rich soils will have to be analysed in future studies.

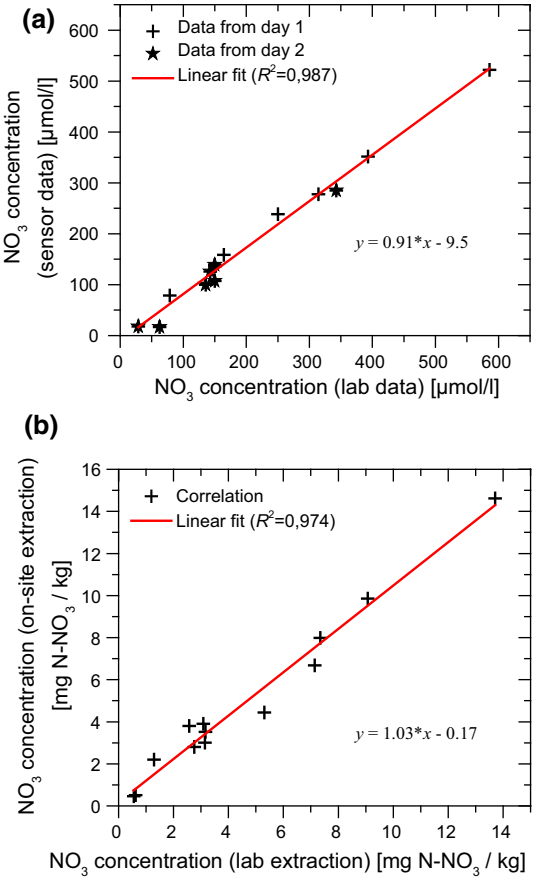

Fig. 7 **a** Fit of C_{NO_3} data from sensor and laboratory measured in the same on-site soil sample extract. **b** Fit of C_{NO_3} data from sensor after on-site extraction and laboratory analysis with standard extraction procedure

K^+ measurement

All sensor values of C_K were above LOQ.

Sensor versus laboratory measurements of on-site soil sample extracts: Sensor and laboratory values of C_K measured in the on-site soil sample extracts are compared in Fig. 8a. The strong correlation with $R^2 = 0.985$ demonstrates the potential of the sensor for on-site measurements of water-extractable K.

On-site vs laboratory extraction: The water-extractable K^+ concentrations measured with the sensor did not correlate with K^+ concentrations obtained with the standard laboratory extraction (see Fig. 8b). The reason is the binding of K^+ ions to soil particles, which demands far stronger extraction solutions like CAL in future studies.

PO_4 measurements

The extracted amount of PO_4 was not sufficient for quantification with the sensor as all C_{PO_4} values were below LOQ. Thus, the use of stronger PO_4 extractants (e.g. CAL) and a sensitivity increase has to be achieved in future studies.

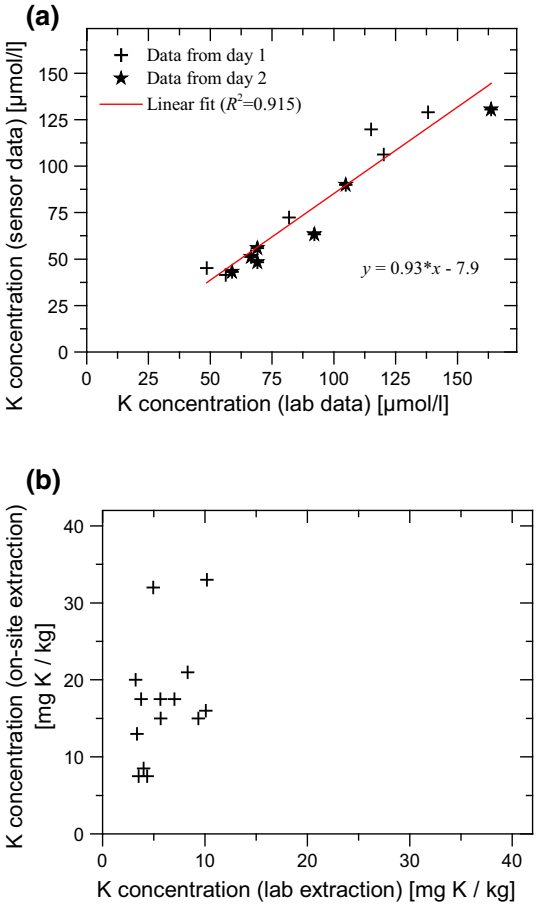

Fig. 8 **a** *Sensor data verification for K* K sensor data versus laboratory data, measured with the same soil sample extract obtained by on-site extraction. **b** *On-site extraction verification* K concentrations measured with the sensor after on-site extraction versus K concentration from standard laboratory extraction and laboratory analysis

Additional ions SO_4^{2-}, Na^+ and Ca^{2+}

The electropherograms of Fig. 6 contain peak signals of SO_4^{2-}, Na^+, Ca^{2+} ions which would allow quantification of these soil components as well. Although no quantitative analysis was done in this study, this highlights the potential of the sensor for multi-ion soil analysis.

Conclusions

The application of a micro-chip capillary electrophoresis sensor device for soil nutrient analysis was demonstrated for the first time. A central advantage of this sensor is the general sensitivity to ions in liquids. The device is thus especially sensitive to the four plant macro-nutrient ions NO_3, NH_4, K and PO_4. A strong linearity of the sensor calibration for these four ions was found. Although the measurement time was high compared to on-the-go sensors from the literature, the sensor can detect the most important plant nutrients in their short term available form. Nutrient measurements near the field with simplified extraction would strongly simplify the sample logistics (no cooled transport) and would reduce analysis costs in comparison to standard laboratory analysis. With these characteristics, the presented instrument could become a candidate for a mobile sensor for plant available macro-nutrients.

The sensor was tested in on-site measurements on samples of different soil type with water extraction. This extraction was chosen for the first sensor test as no ions that can interfere with the sensor reading are added to the samples.

From the measurements, it is concluded that:

(1) The sensor resolved the entire NO_3 concentration range of the analysed DIW extracts; the values were strongly correlated to laboratory data measured in the same extracts. Also a plot of the sensor data versus laboratory data obtained with a standard extraction procedure yielded a strong correlation. This demonstrates the potential of the sensor for on-site NO_3 measurements and of the on-site extraction procedure for NO_3 extraction. These findings have to be tested with larger statistics in future.

(2) NH_4 concentrations were mainly too low to be quantified by the sensor. They amounted on average to 6.4 % of the mineral N concentration (sum of NO_3 and NH_4 concentrations). Thus NH_4 was only a minor source of plant available nitrogen and consequently of minor importance for soil fertility estimations. Measurements in NH_4 rich soils have to be performed in the future for a characterisation of the sensor in the analysis of this ion.

(3) Water soluble K amounts measured with the sensor correlated strongly with laboratory measurements of the same extracts. The sensor is therefore useful for on-site measurement of water-extractable K. In contrast, it was confirmed, that the sensor data after DIW on-site extraction did not correspond to data after standard laboratory extraction.

(4) In PO_4 measurements, the water-extracted amount of all samples was too low to be quantified by the sensor. Future investigations must therefore focus on the use of stronger extraction solutions and sensitivity enhancements of the sensor.

(5) The electropherograms of DIW soil sample extracts resolved peak signals corresponding to SO_4, Na, Ca ions. Future studies, focusing on the evaluation of these parameters, could make the device a versatile multi-ion analysis tool.

In summary, future studies have to focus on tests of the sensor with strong extraction solutions for NH_4, K and PO_4. The major challenge will be the separation of nutrients from the high concentrated background of extraction ions. Further measurements with a wider nutrient concentration range (e.g. due to variations between spring and autumn) have to prove the sensor functionality with higher statistical significance.

Acknowledgments The authors gratefully acknowledge the funding from the European Union in the framework of the Optifert project (FP7-OPTIFERT-286772) (http://www.optifert.eu/). Thanks to P. Svasek and E. Svasek for the support with the chip production, S. Mahlknecht and C. Dumhart for the development of the detection electronics, E. Pirker for technical support and Evonik Industries AG for providing the 55 μm PMMA sheets.

References

An, X., Li, M., Zheng, L., Liu, Y., & Sun, H. (2014). A portable soil nitrogen detector based on NIRS. *Precision Agriculture, 15*(1), 3–16.

Artigas, J., Beltran, A., Jimenez, C., Baldi, A., Mas, R., Dominguez, C., et al. (2001). Application of ion sensitive field effect transistor based sensors to soil analysis. *Computers and Electronics in Agriculture, 31*, 281–293.

Becker, H., Mühlberger, H., Hoffmann, W., Clemens, T., Klemm, R., Gärtner, C. (2008). Portable CE system with contactless conductivity detection in an injection molded polymer chip for on-site food analysis. *Proceedings of SPIE 6886, Microfluidics, BioMEMS, and Medical Microsystems VI* (pp. 68860C-1–68860C-7). Bellingham, USA: SPIE.

Bharadwaj, R., & Santiago, J. G. (2005). Dynamics of field-amplified sample stacking. *Journal of Fluid Mechanics, 543*(1), 57–92.

Bindraban, P. S., Stoorvogel, J. J., Jansen, D. M., Vlaming, J., & Groot, J. J. R. (2000). Land quality indicators for sustainable land management: proposed method for yield gap and soil nutrient balance. *Agriculture, Ecosystems & Environment, 81*(2), 103–112.

Birrell, S., Hummel, J. (1997). Multi-sensor ISFET system for soil analysis. *Proceedings of the First European Conference on Precision Agriculture* (pp. 459–468). Oxford, UK: Bios Scientific Publishers.

Birrell, S. J., & Hummel, J. W. (2001). Real-time multi-ISFET/FIA soil analysis system with automatic sample extraction. *Computers and Electronics in Agriculture, 32*(1), 45–67.

Christy, C., Drummond, P., Lund, E. (2006). Precision agriculture applications of an on-the-go soil reflectance sensor. *Proceedings of the Eighth International Conference on Precision Agriculture*. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.500.9082&rep=rep1&type=pdf. Accessed 24 Jan 2016.

Coltro, W. K. T., Lima, R. S., Segato, T. P., Carrilho, E., de Jesus, D. P., do Lago, C. L., et al. (2012). Capacitively coupled contactless conductivity detection on microfluidic systems—ten years of development. *Analytical Methods, 4*, 25–33.

Ewing, A. G., Wallingford, R. A., & Olefirowicz, T. M. (1989). Capillary electrophoresis. *Analytical Chemistry, 61*(4), 292A–303A.

Fercher, G., Haller, A., Smetana, W., & Vellekoop, M. J. (2010a). Ceramic capillary electrophoresis chip for the measurement of inorganic ions in water samples. *Analyst, 135*, 965–970.

Fercher, G., Haller, A., Smetana, W., & Vellekoop, M. J. (2010b). End-to-end differential contactless conductivity sensor for microchip capillary electrophoresis. *Analytical Chemistry, 82*, 3270–3275.

Fracassi da Silva, J. A., & do Lago, C. L. (1998). An oscillometric detector for capillary electrophoresis. *Analytical Chemistry, 70*, 4339–4343.

Gärtner, C., Hoffmann, W., Demattio, H., Clemens, T., Klotz, M., Klemm, R., Becker, H. (2009). Portable integrated capillary-electrophoresis system using disposable polymer chips with capacitively coupled contactless conductivity detection for on-site analysis of foodstuff. *Proceedings of SPIE 7315, Sensing for Agriculture and Food Quality and Safety* (pp. 731505–731506). Bellingham, USA: SPIE.

Guijt-van Duijn, R. M., Evenhuis, C. J., Macka, M., & Haddad, P. R. (2004). Conductivity detection for conventional and miniaturised capillary electrophoresis systems. *Electrophoresis, 25*(23–24), 4032–4057.

Ho, C.-H., & Tsay, Y.-F. (2010). Nitrate, ammonium, and potassium sensing and signaling. *Current Opinion in Plant Biology, 13*(5), 604–610.

Howald, M., Elsenbeer, H., Laczko, E., & Schlunegger, U. P. (1995). Capillary electrophoresis—a fast and universal tool in soil analysis. *Journal of Analytical Sciences, Methods and Instrumentation, 2*(4), 170–175.

Karlinsey, J. M. (2012). Sample introduction techniques for microchip electrophoresis: a review. *Analytica Chimica Acta, 725*, 1–13.

Kim, H.-J., Hummel, J. W., Sudduth, K. A., & Motavalli, P. P. (2007). Simultaneous analysis of soil macronutrients using ion-selective electrodes. *Soil Science Society of America Journal, 71*, 1867–1877.

Kodaira, M., & Shibusawa, S. (2013). Using a mobile real-time soil visible-near infrared sensor for high resolution soil property mapping. *Geoderma, 199*, 64–79.

Kubáň, P., & Hauser, P. C. (2009). Ten years of axial capacitively coupled contactless conductivity detection for CZE—a review. *Electrophoresis, 30*(1), 176–188.

Kubán, P., Nguyen, H., Macka, M., Haddad, P., & Hauser, P. C. (2007). New fully portable instrument for the versatile determination of cations and anions by capillary electrophoresis with contactless conductivity detection. *Electroanalysis, 19*, 2059–2065.

Kuhr, W. G. (1990). Capillary electrophoresis. *Analytical Chemistry, 62*(12), 403R–414R.

Laugere, F., Guijt, R. M., Bastemeijer, J., van der Steen, G., Berthold, A., Baltussen, E., et al. (2003). On-chip contactless four-electrode conductivity detection for capillary electrophoresis devices. *Analytical Chemistry, 75*(2), 306–312.

O'Flaherty, B. D., Barry, E. F., & Cholli, A. L. (2000). A rapid soil nutrient sensor device based on capillary zone electrophoresis. *Journal of Environmental Science and Health, Part A, 35*, 189–201.

Oreshkin, N. (1980). Extraction of mobile forms of phosphorus and potassium by the Egner–Riehm–Domingo method. *Agrokhimiia, 8*, 135–138.

Puchberger-Enengl, D., Bipoun, M., Smolka, M., Krutzler, C., Keplinger, F., Vellekoop, M.J. (2013). Hydrogel plug for independent sample and buffer handling in continuous microchip capillary electrophoresis. *Proceedings of SPIE, Smart Sensors, Actuators, and MEMS VI* (pp. 87631B–87631B-6). Bellingham, USA: SPIE.

Roberts, D. C., Brorsen, B. W., Solie, J. B., & Raun, W. R. (2013). Is data needed from every field to determine in-season precision nitrogen recommendations in winter wheat? *Precision Agriculture, 14*, 245–269.

Roth, G. W., Beegle, D. B., Fox, R. H., Toth, J. D., & Piekielek, W. P. (1991). Development of a quicktest kit method to measure soil nitrate. *Communications in Soil Science and Plant Analysis, 22*, 191–200.

Schirrmann, M., Gebbers, R., & Kramer, E. (2013). Performance of automated near-infrared reflectance spectrometry for continuous in situ mapping of soil fertility at field scale. *Vadose Zone Journal, 12*(4), 1–10.

Scholefield, D., & Titchen, N. (1995). Development of a rapid field test for soil mineral nitrogen and its application to grazed grassland. *Soil Use and Management, 11*, 33–43.

Shaw, R., Williams, A., Miller, A., & Jones, D. (2013). Assessing the potential for ion selective electrodes and dual wavelength UV spectroscopy as a rapid on-farm measurement of soil nitrate concentration. *Agriculture, 3*, 327–341.

Shi, T., Cui, L., Wang, J., Fei, T., Chen, Y., & Wu, G. (2012). Comparison of multivariate methods for estimating soil total nitrogen with visible/near-infrared spectroscopy. *Plant and Soil, 366*(November), 363–375.

Shibusawa, S. (2003). On-line real time soil sensor. *IEEE/ASME International Conference on Advanced Intelligent Mechatronics 2003* (vol. 2, pp. 1061-1066). New York, USA: IEEE.

Sibley, K.J., Brewster, G.R., Astatkie, T., Adsett, J.F., Struik, P.C. (2010). In-field measurement of soil nitrate using an ion-selective electrode. *Advances in Measurement Systems*, *InTech Open* (pp. 1–27). http://cdn.intechweb.org/pdfs/9958.pdf. Accessed 24 Jan 2016.

Smolka, M., Puchberger-Enengl, D., Bipoun, M., Fercher, G., Klasa, A., Krutzler, C., et al. (2013). A new injection method for soil nutrient analysis in capillary electrophoresis. *Proceedings of SPIE 8763, Smart Sensors, Actuators, and MEMS VI* (pp. 87631C-1–87631C-7). Bellingham, USA: SPIE.

Swinney, K., & Bornhop, D. J. (2000). Detection in capillary electrophoresis. *Electrophoresis, 21*(7), 1239–1250.

Tanyanyiwa, J., & Hauser, P. C. (2002). High-voltage capacitively coupled contactless conductivity detection for microchip capillary electrophoresis. *Analytical Chemistry, 74*(24), 6378–6382.

Vandaveer, W. R., Pasas-Farmer, S., Fischer, D. J., Frankenfeld, C. N., & Lunte, S. M. (2004). Recent developments in electrochemical detection for microchip capillary electrophoresis. *Electrophoresis, 25*, 21–22.

Viscarra Rossel, R. A., & Behrens, T. (2010). Using data mining to model and interpret soil diffuse reflectance spectra. *Geoderma, 158*(1–2), 46–54.

Viscarra Rossel, R. A., Walvoort, D. J. J., Mc Bratney, A. B., Janik, L. J., & Skjemstad, J. O. (2006). Visible, near infrared, mid infrared or combined diffuse reflectance spectroscopy for simultaneous assessment of various soil properties. *Geoderma, 131*, 59–75.

Vohland, M., Ludwig, M., Thiele-Bruhn, S., & Ludwig, B. (2014). Determination of soil properties with visible to near- and mid-infrared spectroscopy: effects of spectral variable selection. *Geoderma, 223–225*(November), 88–96.

Wetselaar, R., Smith, G. D., & Angus, J. F. (1998). Field measurement of soil nitrate concentrations. *Communications in Soil Science and Plant Analysis, 29*, 729–739.

Zemann, A. J., Schnell, E., Volgger, D., & Bonn, G. K. (1998). Contactless conductivity detection for capillary electrophoresis. *Analytical Chemistry, 70*, 563–567.