

# Isotopic signatures of production and uptake of H<sub>2</sub> by soil

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**Abstract:** Molecular hydrogen ( $H_2$ ) is the second most abundant reduced trace gas (after methane) in the atmosphere, but its biogeochemical cycle is not well understood. Our study focuses on the soil production and uptake of  $H_2$  and the associated isotope effects. Air samples from a grass field and a forest site in the Netherlands were collected using soil chambers. The results show that uptake and emission of  $H_2$  occurred simultaneously at all sampling sites, with strongest emission at the grassland sites where clover ( $N_2$  fixing legume) was present. The  $H_2$  mole fraction and deuterium content were measured in the laboratory to determine the isotopic fractionation factor during  $H_2$  soil uptake ( $\alpha_{soil}$ ) and the isotopic signature of  $H_2$  that is simultaneously emitted from the soil ( $\delta D_{soil}$ ). By considering all net-uptake experiments, an overall fractionation factor for deposition of  $\alpha_{soil} = k_{HD} / k_{HH} = 0.945 \pm 0.004$  (95% CI) was obtained. The difference in mean  $\alpha_{soil}$  between the forest soil  $0.937 \pm 0.008$  and the grassland  $0.951 \pm 0.025$  is not statistically significant. For two experiments, the removal of soil cover increased the deposition velocity ( $v_d$ ) and  $\alpha_{soil}$  simultaneously, but a general positive correlation between  $v_d$  and  $\alpha_{soil}$  was not found in this study. When the data are evaluated with a model of simultaneous production and uptake, the isotopic composition of  $H_2$  that is emitted at the grassland site is calculated as  $\delta D_{soil} = (-530 \pm 40) \text{‰}$ . This is less deuterium-depleted than what is expected from isotope equilibrium between  $H_2O$  and  $H_2$ .

## 1. Introduction

H<sub>2</sub> is considered as alternative energy carrier to replace fossil fuels in the future. However, the environmental and climate impact of a potential widespread use of H<sub>2</sub> is still under assessment. Several studies suggested that the atmospheric H<sub>2</sub> mole fraction might increase substantially in the future due to the leakage during production, storage, transportation and use of H<sub>2</sub>, which could significantly affect atmospheric chemistry (Schultz et al., 2003; Tromp et al., 2003; van Ruijven et al., 2011; Warwick et al., 2004).

In the troposphere, H<sub>2</sub> has a mole fraction of about 550 parts per billion (ppb = nmol mol<sup>-1</sup>) and a lifetime of around 2 years (Novelli et al., 1999; Price et al., 2007; Xiao et al., 2007; Pieterse et al., 2011; 2013). H<sub>2</sub> can affect atmospheric chemistry and composition in several ways. Firstly, it increases the lifetime of the greenhouse gas methane (CH<sub>4</sub>) via its competing reaction with the hydroxyl radical (OH) (Schultz et al., 2003; Warwick et al., 2004). Additionally, H<sub>2</sub> affects air quality because it is an ozone (O<sub>3</sub>) precursor and indirectly increases the lifetime of the air pollutant carbon monoxide (CO) through competition for OH. In the stratosphere, H<sub>2</sub>O that is produced through the oxidation of H<sub>2</sub> increases humidity, which can result in increased formation of polar

stratospheric clouds and O<sub>3</sub> depletion (Tromp, et al., 2003), but this effect may be weaker than estimated initially (Warwick et al. 2004; Vogel et al., 2012).

The main sources of tropospheric H<sub>2</sub> are the oxidation of CH<sub>4</sub> and non-methane hydrocarbons (NMHC) (48%), biomass burning (19%), fossil fuel combustion (22%) and biogenic N<sub>2</sub> fixation in the ocean (6%) and on land (4%), while the main sinks are soil uptake (70%) and oxidation by OH (30%) (Pieterse et al, 2013).

The biogenic soil sink of H<sub>2</sub> is the largest and most uncertain term in the global atmospheric H<sub>2</sub> budget. Conrad and Seiler (1981) assumed that the soil uptake of atmospheric H<sub>2</sub> is most likely due to consumption by abiotic enzymes, since there were no soil microorganisms known to be able to fix H<sub>2</sub> at the low atmospheric mole fraction at that time. This remained the basic hypothesis of many further soil uptake studies (Conrad et al., 1983; Conrad and Seiler, 1985; Ehhalt and Rohrer, 2011; Guo and Conrad, 2008; Häring et al., 1994; Smith-Downey et al., 2006). However, Constant et al. (2008a) were first to identify an aerobic microorganism (*Streptomyces* sp. PCB7) that can consume H<sub>2</sub> at tropospheric ambient mole fractions, and suggested that active metabolic cells could be responsible for the soil uptake of H<sub>2</sub> rather than extracellular enzymes. Further studies showed that uptake activity at ambient H<sub>2</sub> level is widespread among the streptomycetes (Constant et al., 2010) and it was postulated that high



affinity  $H_2$ -oxidizing bacteria are the main biological agent responsible for the soil uptake of atmospheric  $H_2$  (Constant et al., 2011). Khdhiri et al. (2015) suggested that the relative abundance of high affinity  $H_2$ -oxidation bacteria and soil carbon content could be used as predictive parameters for the  $H_2$  oxidation rate. Determining the dominant mechanism of the  $H_2$  soil uptake activity is still an active area of research.

It has been shown that soil uptake of  $H_2$  can coexist with soil production (Conrad, 1994).  $H_2$  is produced in the soil during  $N_2$  fixation (e.g. by bacteria living symbiotically in the roots of legumes such as clover or beans) and dark fermentation. Although the  $H_2$  produced in the soil by e.g.  $N_2$  fixation can be largely consumed within the soil, a significant amount of  $H_2$  escapes to the atmosphere (Conrad and Seiler, 1979; 1980). Conrad and Seiler (1980) estimated that 2.4 to 4.9 Tg  $a^{-1}$  of  $H_2$  is emitted into the atmosphere through  $N_2$  fixation on land.

One approach to better understand the sources and sinks of  $H_2$  is to investigate the isotopic fractionation processes involved, which act as a fingerprint for  $H_2$  emitted from different sources or destroyed by different sinks. The isotopic composition of  $H_2$  is expressed as:

$$\delta(\text{D}, \text{H}_2) = \frac{R_{\text{sa}}}{R_{\text{VSMOW}}} - 1$$

107  
 108 where  $R_{\text{sa}}$  is the D/H ratio of the sample  $\text{H}_2$  and  $R_{\text{VSMOW}} = (155.76 \pm 0.8)$  parts per  
 109 million ( $\text{ppm} = \text{mmol mol}^{-1}$ ) is the same ratio of the standard material, Vienna  
 110 Standard Mean Ocean Water (VSMOW) (De Wit et al., 1980; Gonfiantini et al.,  
 111 1993). For brevity, we will use the notation  $\delta\text{D}$  ( $=\delta\text{D}(\text{D}, \text{H}_2)$ ) throughout the  
 112 rest of this paper. The  $\delta\text{D}$  values are usually given in per mill ( $\text{‰}$ ). Recent  
 113 studies showed that the global mean  $\delta\text{D}$  value of atmospheric  $\text{H}_2$  is about  $+130 \text{ ‰}$   
 114 (Batenburg et al., 2011; Gerst et al., 2000, 2001; Rice et al., 2010).

115  
 116 The  $\text{HH}$  molecule is consumed preferentially over  $\text{HD}$  during both  $\text{OH}$   
 117 oxidation and soil uptake, with  $\text{OH}$  oxidation causing a much stronger isotope  
 118 fractionation effect. Only a few studies have investigated the soil uptake of  $\text{H}_2$   
 119 with isotope techniques. Gerst and Quay (2001) carried out field experiments in  
 120 Seattle, United States and found  $\alpha_{\text{soil}} (= k_{\text{HD}}/k_{\text{HH}})$  to be  $0.943 \pm 0.024$  ( $1\sigma$ ). Note  
 121 that  $k_{\text{HD}}$  and  $k_{\text{HH}}$  are removal rate constants for  $\text{HD}$  and  $\text{HH}$  respectively. Rahn  
 122 et al. (2002a) collected air samples from four forest sites in ecosystems of  
 123 different ages in Alaska, United States, in July 2001, and obtained a similar  
 124 average value ( $0.94 \pm 0.01$ ). They suggested that  $\alpha_{\text{soil}}$  depends on the forest  
 125 maturity, with smaller fractionation for more mature forests. Since the more  
 126 mature forests showed larger deposition velocity ( $v_d$ ) of  $\text{H}_2$ , they further

suggested that lower uptake rates involve greater isotopic fractionation ( $\alpha_{\text{soil}}$  further from 1) than fast uptake rates. Rice et al. (2011) performed deposition experiments in Seattle and found  $\alpha_{\text{soil}}$  varying from 0.891 to 0.976, with a mean of 0.934. They found  $\alpha_{\text{soil}}$  to be correlated with  $v_d$ , with smaller isotope effects ( $\alpha_{\text{soil}}$  closer to 1) occurring at higher  $v_d$ , which agreed with the suggestion by Rahn et al. (2002a). In addition, unpublished experiments from Rahn et al. (2005) yielded  $\alpha_{\text{soil}} = 0.89 \pm 0.03$  in three upland ecosystems that were part of an Alaskan fire chronosequence. The data suggest that variability in the soil/ecosystem affects  $\alpha_{\text{soil}}$  but no significant variability of  $\alpha_{\text{soil}}$  with season was detected. Hitherto, only  $\alpha_{\text{soil}}$  values from studies in Seattle and Alaska are available, and values from other locations and ecosystems are needed to learn more about the factors influencing  $\alpha_{\text{soil}}$ .

The  $\delta D$  of  $H_2$  from various surface sources has been reported as about -290 ‰ for biomass burning (Gerst and Quay, 2001; Haumann et al., 2013) and between -200 ‰ and -360 ‰ for fossil fuels combustion (Rahn et al., 2002b; Vollmer et al., 2012). So far no field studies have determined the isotopic composition of the  $H_2$  emitted from soil. Two laboratory studies examined the isotopic signature of  $H_2$  produced from  $N_2$  fixation. Luo et al. (1991) reported a fractionation factor  $\alpha_{H_2/H_2O} = R(D/H, H_2)/R(D/H, H_2O) = 0.448 \pm 0.001$  between the  $H_2$  produced from  $N_2$  fixation and the  $H_2O$  used to grow the  $N_2$ -fixing bacteria for *Synechococcus* sp. and  $0.401 \pm 0.002$  for *Anabaena* sp., respectively.

Walter et al. (2012) reported  $\alpha_{\text{H}_2/\text{H}_2\text{O}} = 0.363 \pm 0.019$  for the  $\text{N}_2$ -fixing rhizobacterium *Azospirillum brasiliensis*. It has been proposed that microbiological  $\text{H}_2$  consumption and production could modify the thermal isotopic equilibrium between  $\text{H}_2$  and  $\text{H}_2\text{O}$  in low-temperature hydrothermal fluids (Kawagucci et al., 2010). Compared to the surface sources,  $\text{H}_2$  produced from  $\text{CH}_4$  and NMHC oxidation is isotopically strongly enriched in deuterium, with  $\delta\text{D}$  between +120 and +180 ‰ (Rahn et al., 2003; Röckmann et al. 2003a, Pieterse et al., 2011).

Here we report measurements of the isotopic fractionation factors of  $\text{H}_2$  during soil deposition at two different sites in the Netherlands, a forest and a grassland site. For the grassland site we also determine the apparent isotopic composition of the  $\text{H}_2$  that was simultaneously emitted from the soil during the experiment.

## 2. Methods

### 2.1 Sampling

Air samples were collected from a soil chamber at two locations in the Netherlands (Fig. 1): a grass field around the Cabauw tall tower (51°58' N,

170 4°55' E) and a forest site near Speuld (52°13' N, 5°39' E). Two types of ground  
171 cover (grass with and without clover) were sampled at Cabauw, while three  
172 types of forest (Douglas fir, beech and spruce) were selected in Speuld. More  
173 information about the soil and vegetation type can be found in Beljaars and  
174 Bosveld (1997) for the Cabauw site, and in Heij and Erisman (1997) for the  
175 Speuld site.

176

177 Flask samples were filled with air from a soil chamber, using a closed-cycle air  
178 sampler (Fig. 2). The soil chamber consisted of two parts: the chamber body  
179 with a metal base at the bottom that was inserted about 2 cm into the soil, and a  
180 removable transparent lid with two connections for air sampling. The chamber  
181 had a height of 40 cm, an area of 570 cm<sup>2</sup> and a volume of 22.8 L; the air inside  
182 was mixed by a fan. The sampler could hold four flasks installed in series,  
183 which could be bypassed independently; the flow and pressure in the flasks  
184 were controlled. The air was dried using Mg(ClO<sub>4</sub>)<sub>2</sub>. After passing through the  
185 flasks the air was returned to the soil chamber, which kept the pressure inside  
186 the chamber approximately constant during sampling.

187

188 Air samples were collected from the chamber in 1 L glass flasks at 0, 10, 20 and  
189 30 minutes after closing the chamber (time interval changed to 5 minutes in  
190 Speuld because of the faster uptake). The gas flasks (Normag, Ilmenau,  
191 Germany) were made of borosilicate glass 3.3 with O-ring-sealed stopcocks

192 made of PCTFE (Kel-F) and covered with a dark hose. Thorough tests have  
193 demonstrated that air samples with typical trace gas content are stable in these  
194 flasks (Rothe et al., 2004). In the beginning, the whole sampling unit (all lines,  
195 connections and flasks) was flushed with ambient air for about 10 minutes at a  
196 flow rate of  $2 \text{ L min}^{-1}$  and a pressure of 100 kPa, with all flasks open and the  
197 chamber lid open. This initial flushing process was designed to fill the flasks  
198 with background air. The air pressure inside the flasks was increased to 200 kPa  
199 (180 kPa for Speuld samples) by adjusting the flow control valve and the valves  
200 on two pressure gauges (Fig. 2) before chamber closing and then maintained  
201 constant during the whole sampling time. The flow rate was maintained at  $2 \text{ L}$   
202  $\text{min}^{-1}$  at ambient pressure and temperature with a rotameter and the pressure  
203 inside the chamber was maintained at 100 kPa during the whole sampling time.  
204 The temperature was not recorded during the sampling. After the initial flushing,  
205 the first flask was closed and then the chamber was closed as well. Afterwards,  
206 the air was flushed from the chamber through three flasks (the first flask was  
207 by-passed) and back to the chamber. After 10, 20 and 30 minutes, the second,  
208 third and fourth flasks were closed.

209  
210 A total of 36 sets of air samples were collected in Cabauw during summer (June,  
211 July and August) 2012 and 12 sets were collected in Speuld in September 2012.  
212 Each set contains four air samples. In total, 186 valid samples were analyzed for  
213  $\text{H}_2$  mole fraction and its deuterium content (6 were lost during sampling,

transportation and measurement). All the Speuld samples and about half of the Cabauw samples were further used for analysis in this study. The reason why 50% of the Cabauw experiments were not used is that these experiments showed neither strong H<sub>2</sub> emission nor H<sub>2</sub> uptake and the isotopic signals were weak. Most experiments were conducted with the 22.8 L volume soil chamber as described above, while 10 experiments were conducted with a larger automated soil chamber with a volume of 125 L and a height of 22.5 cm.

## **2.2 Laboratory determination of H<sub>2</sub> mole fraction and deuterium content of air samples**

The mole fraction and the  $\delta D$  of H<sub>2</sub> were measured with a gas chromatography isotope ratio mass spectrometry (GC/IRMS) setup (Rhee et al., 2004). For H<sub>2</sub> mole fractions, the laboratory working standards are linked to the MPI-2009 scale (Jordan and Steinberg, 2011). The  $\delta D$  values of the laboratory reference gases are indirectly linked to mixtures of synthetic air with H<sub>2</sub> of known isotopic composition, certified by Messer Griesheim, Germany (Batenburg et al., 2011). Most of the samples collected from Cabauw were measured within two months after sampling, while the samples from Speuld were kept in a dark storage room for around four months before measurement.

235

236 The operational principle of the GC/IRMS system is to separate H<sub>2</sub> from the air  
237 matrix at low temperature (about 36 K) and measure the HH and HD content  
238 with a mass spectrometer. The measurement includes four main steps:

239

240 (1) A glass sample volume (750 ml) is evacuated and subsequently filled with  
241 sample air to approximately 700 mbar. This volume is then exposed to a cold  
242 head (36 K) of a closed-cycle helium compressor for 9 minutes. During this  
243 stage, all gases except H<sub>2</sub>, helium (He) and neon (Ne) condense.

244

245 (2) The remainder in the headspace of the cold head and sample volume is then  
246 flushed with He carrier gas to a pre-concentration trap where H<sub>2</sub> is collected on  
247 a 25 cm long, 1/8 inch OD (outside diameter) stainless steel tube filled with fine  
248 grains (0.2 to 0.5 mm) of 5 Å molecular sieve, for 20 minutes. The pre-  
249 concentration trap is cooled down to the triple point of nitrogen (63 K) by  
250 keeping it in a liquid N<sub>2</sub> reservoir that is further cooled down by pumping on the  
251 gas phase.

252

253 (3) After the collection of H<sub>2</sub>, the pre-concentration trap is warmed up to release  
254 the absorbed H<sub>2</sub>, which is then cryo-focused for 4 minutes on a capillary (25 cm  
255 long, 0.32 mm ID (inside diameter)) filled with 5 Å molecular sieve at 77 K.  
256 After that, the cryo-focus trap is warmed up to ambient temperature and the H<sub>2</sub>



sample is flushed with He carrier gas onto the GC column (5 Å molecular sieve,  $\approx 323$  K) where  $H_2$  is chromatographically purified from potential remaining interferences.

(4) In the end, the purified  $H_2$  is carried by the He carrier gas via an open split interface (Röckmann et al., 2003b) into the IRMS for D/H ratio determination.

More details about the GC/IRMS system and measurement steps can be found in Rhee et al. (2004) and Röckmann et al. (2010). The data correction procedures and isotope calibration are similar to those described in Batenburg et al. (2011). Four reference gases were used to determine the  $\delta D$  values of the samples. Two of them (Ref-1 and Ref-2) with  $\delta D$  values of  $(+207.0 \pm 0.3) \text{‰}$  and  $(+198.2 \pm 0.5) \text{‰}$  were calibrated and used previously in Batenburg et al. (2011). The other two new reference gases (Ref-3 and Ref-4) were calibrated versus Ref-1 and Ref-2. The  $\delta D$  value of Ref-3 was  $(-183 \pm 2.4) \text{‰}$ . Ref-4 was a frequently measured reference gas that was measured usually about 5 times per sequence of measurement, while other three reference gases were measured about 1 to 3 times per sequence of measurement. The  $\delta D$  value of Ref-4 dropped linearly with time from  $-115 \text{‰}$  to  $-157 \text{‰}$  between 1 Jun 2012 and 15 Feb 2013, while the other three reference gases were stable.

## 2.3 Non-linearity of the GC/IRMS system

Ideally, the  $\delta D$  of  $H_2$  measured with the GC/IRMS should not depend on the total amount of  $H_2$  used for analysis, but in practice a dependence of the isotopic composition on the amount of  $H_2$  is observed for low mole fractions. This is called non-linear behavior, and it is a particularly severe limitation for soil uptake studies, since the mole fraction in such samples can decrease by more than an order of magnitude. For comparison, in ambient background air the  $H_2$  mole fraction variations are usually no more than 20%.

Experiments were carried out with different quantities of air from various laboratory reference bottles with known  $\delta D$  to determine a suitable correction for the non-linear behavior. The measured  $\delta D$  increases with the mass 2 sample peak area, which is proportional to the  $H_2$  quantity in the sample. In the peak area range of 0.2 Vs to 1 Vs this relation can be parameterized by a logarithmic function  $\delta D = 54.6 \ln (\text{peak area}/Vs) \text{ ‰}$ , which is used as correction function for the measurements at low peak areas (Fig. 3). The linearity correction introduces an additional uncertainty due to uncertainties in the logarithmic fit, particularly at low peak areas. The total assigned uncertainty for each measurement is calculated from the analytical and fitting uncertainty, as a function of peak area (Fig. 4). It is 2 ‰ for  $\ln (\text{peak area}/Vs)$  of 1.5 or more

(equivalent to more than 600 ppb H<sub>2</sub> in an air sample), but increases to 32 ‰ when ln (peak area/Vs) drops to -1.6 (≈ 20 ppb H<sub>2</sub> in air sample). In total, the δD results of 18 Speuld samples that were measured at these low peak areas were corrected with this linearity correction. Possible additional systematic errors (a few ‰) may arise from uncertainties in the initially assigned δD values of the commercial calibration gases, changes of these values in the process of creating calibration mixtures with near-ambient H<sub>2</sub> concentration, and the calibration measurements themselves (Batenburg et al., 2011).

## 2.4 Data evaluation

Assuming first order kinetics for H<sub>2</sub> removal and a constant production rate  $P$  over the course of a deposition experiment, the time evolution of the mole fraction  $c$  of non-deuterated H<sub>2</sub> (HH) inside the soil chamber can be expressed as:

$$\frac{dc}{dt} = P - kc \quad (1)$$

where  $k$  is the first order uptake rate constant of HH. For well-mixed air in the chamber,  $k = v_d/h$ , where  $v_d$  is the gross deposition velocity of H<sub>2</sub> and  $h$  is the

chamber height. The gross deposition velocity is the deposition velocity corrected for production, which is different from the net deposition velocity reported in some studies in the past that showed the effective uptake of  $H_2$  from the atmosphere. The solution of Eq. (1) is of the form:

$$c = (c_i - c_e)e^{-kt} + c_e \quad (2)$$

where  $c$ ,  $c_i$  and  $c_e (= P/k)$  are the mole fractions of HH at time  $t$ , initially and at equilibrium, respectively. Therefore,  $P$  and  $k$  can be obtained by fitting an exponential function to the time evolution of HH inside the chamber. Similarly, we can obtain  $P'$  and  $k'$  from the time evolution of HD.

$$c' = (c'_i - c'_e)e^{-k't} + c'_e \quad (3)$$

where  $c'$ ,  $c'_i$ ,  $c'_e (= P'/k')$ ,  $P'$  and  $k'$  are the corresponding parameters for HD.

Equations (2) and (3) constitute the mass balance model that we used to analyze our data. When  $k$ ,  $k'$ ,  $P$  and  $P'$  have been determined,  $\alpha_{\text{soil}}$  and  $\delta D_{\text{soil}}$  can be calculated simply as:

$$\alpha_{\text{soil}} = \frac{k'}{k} \quad (4)$$

337

$$\delta D_{\text{soil}} = \frac{P'/P}{2R_{\text{VSMOW}}} - 1 \quad (5)$$

338

339 However, fitting an exponential curve to only four sample data yields relatively  
 340 large errors for  $k, k', P$  and  $P'$ , which propagate to large errors for  $\alpha_{\text{soil}}$  and  $\delta D_{\text{soil}}$   
 341 if they are determined directly from Eqs (4-5).

342

343 In Rice et al. (2011), Equations (2) and (3) were combined to calculate  $\alpha_{\text{soil}}$  in  
 344 the presence of both source and sink of  $\text{H}_2$  using  $c_e$  and  $c_e'$  from the exponential  
 345 fits:

346

$$\ln \frac{c' - c_e'}{c_i' - c_e'} = \frac{k'}{k} \ln \frac{c - c_e}{c_i - c_e} \quad (6)$$

347

348  $\alpha_{\text{soil}} = k'/k$  can be obtained by plotting  $\ln \frac{c' - c_e'}{c_i' - c_e'}$  versus  $\ln \frac{c - c_e}{c_i - c_e}$  and fitting a linear  
 349 function. In the absence of soil emission ( $c_e = c_e' = 0$ ), Eq. (6) collapses to the  
 350 well-known Rayleigh fractionation equation that is used to quantify the isotope  
 351 fractionation during single stage removal processes in the absence of sources.

352

For the high emission measurements, where production overwhelms consumption, we use the relations  $c_e = P/k$  and  $c'_e = P'/k'$ , and obtain  $P'/P$  from the slope of  $c'_e \ln \frac{c'_i - c'_e}{c'_i - c'_e}$  against  $c_e \ln \frac{c - c_e}{c_i - c_e}$ . Then  $\delta D_{\text{soil}}$  is calculated from Eq. (5).

## 2.5 Flask sampling model

The advantage of sampling with the soil chamber system described in Section 2.1 was that the pressure in the soil chamber stayed constant even when several large samples (2 L each) were taken. A disadvantage was that the volume of air inside the flasks (8 L of air in total) was considerable compared to the volume of air inside the soil chamber (22.8 L). This had two effects: (1) A significant part of the air was at each time separated from the chamber and thus from the soil production and uptake. (2) Because of the time lag to flush the samples, the air in a flask was not the same as the air in the chamber at the same time.

We built a flask sampling model to derive correction factors that take into account the influence of the flask sampling system. For a given combination of uptake and production rates, the model simulates the evolution of the  $H_2$  mole fraction in two configurations: the soil chamber alone, and the soil chamber plus four flasks as in our experiments. The model is described in detail in Appendix A. An example of a simulation is shown in Fig. 5. Compared to the situation

without flasks, there is a time lag in the decay of  $H_2$  for both the chamber and the flasks after introducing four flasks in the model. The time lag for the second flask is about 2.5 minutes. It increases to 5 minutes for the third flask and is even longer for the fourth flask.

It is obvious that the sampling process strongly affects the uptake rate  $k_{app}$  and production rate  $P_{app}$  obtained from the direct flask measurements, so we corrected all  $k_{app}$  and  $P_{app}$  values with the correction coefficients derived from this flask sampling model (Appendix A). For a fixed chamber volume, sample pressure, flow rate and time interval of the flask collection that are all recorded for each experiment, the relationship between the actual uptake rate constant  $k_{true}$  and apparent uptake rate constant  $k_{app}$  can be obtained (see Appendix A). Under the same sampling conditions for a fixed value of  $P_{app}$ , the relationship between actual production rate  $P_{true}$  and apparent production rate  $P_{app}$  depends on  $k_{true}$  (Fig 10b).

To evaluate the data, we first applied an exponential fit as in Eq. (2) to the measured HH mole fractions for the four flasks in each experiment and obtained *apparent* values  $k_{app}$ ,  $P_{app}$  and  $c_{e,app}$  from the fit parameters. Then we used the correction factors derived from the flask sampling model to retrieve true values

394  $k_{\text{true}}$  and  $P_{\text{true}}$  from the apparent values  $k_{\text{app}}$  and  $P_{\text{app}}$ . One can obtain  $k'_{\text{true}}$  and  $P'_{\text{true}}$   
 395 by applying the same method to HD mole fractions inside four flasks.

396

397 To determine  $\alpha_{\text{soil}}$ , we plotted  $\ln \frac{c' - c'_{\text{e,app}}}{c'_1 - c'_{\text{e,app}}}$  versus  $\ln \frac{c - c_{\text{e,app}}}{c_1 - c_{\text{e,app}}}$  (Eq.6, Fig. 7) and  
 398 obtained  $\alpha_{\text{soil,app}}$  from the slope of the linear regression. Here,  $c$  and  $c'$  are HH  
 399 and HD mole fractions in each of the four flasks;  $c_1$  and  $c'_1$  are HH and HD  
 400 mole fractions of the first flask;  $c_{\text{e,app}}$  and  $c'_{\text{e,app}}$  are apparent HH and HD  
 401 equilibrium mole fractions obtained from the exponential fits of HH and HD  
 402 mole fractions inside the four flasks. We determined the relationship (Fig. 10c)  
 403 between  $\alpha_{\text{soil,true}}$  and  $\alpha_{\text{soil,app}}$  obtained from  $\ln \frac{c' - c'_{\text{e,app}}}{c'_1 - c'_{\text{e,app}}}$  versus  $\ln \frac{c - c_{\text{e,app}}}{c_1 - c_{\text{e,app}}}$  using the  
 404 flask sampling model (see Appendix A1.3). The correction coefficients for each  
 405 experiment are given in Table 3.

406

407 Similarly, we obtained  $P'_{\text{app}}/P_{\text{app}}$  by plotting  $c'_{\text{e,app}} \ln \frac{c' - c'_{\text{e,app}}}{c'_1 - c'_{\text{e,app}}}$  versus  
 408  $c_{\text{e,app}} \ln \frac{c - c_{\text{e,app}}}{c_1 - c_{\text{e,app}}}$  (Fig. 9), and calculated  $\delta D_{\text{soil,app}}$  by use of Eq. (5). Then we  
 409 retrieved  $\delta D_{\text{soil,true}}$  by use of the flask sampling model (Fig. 10d). The  
 410 corresponding correction coefficients for  $\delta D_{\text{soil,app}}$  for each net-emission  
 411 experiment are shown in Table 3. More information about the retrievals of  
 412  $\alpha_{\text{soil,true}}$  and  $\delta D_{\text{soil,true}}$  can be found in Appendix A.

413



Overall, the sampling effect on  $\delta D_{\text{soil}}$  is small (less than 22‰). This means that the flask sampling system strongly affects the temporal evolution of HH and HD individually (Fig. 5), and the uptake and production rates derived from flask measurements, but the effects on the computed isotopic signature of the source and sink are relatively small. More details and discussion of the flask sampling model corrections are provided in Appendix A.

### 3. Results

#### 3.1 Temporal evolution of H<sub>2</sub>, HD and $\delta D$

Fig. 6 shows examples for the temporal evolution of H<sub>2</sub>, HD and  $\delta D$  in Cabauw and Speuld, with error estimates included. The errors for H<sub>2</sub> and HD are about 4% of the respective mole fraction. The error for  $\delta D$  ranges from 2 ‰ to 17 ‰.

Some of our Cabauw experiments show net soil emission of H<sub>2</sub> (upper panels) and some show net soil uptake (middle panels), while all Speuld experiments show net uptake of H<sub>2</sub> (lower panels). In the Cabauw net emission experiments, the increase in H<sub>2</sub> mole fractions is associated with a strong decrease in  $\delta D$ , showing a strongly depleted H<sub>2</sub> source. However, the net uptake experiments at

Cabauw show also a decrease in  $\delta D$ , albeit smaller. In the Speuld experiments, the uptake of  $H_2$  is much faster; the  $\delta D$  increases in the beginning but then decreases again towards the end of the sampling, when the  $H_2$  mole fractions are low.

As mentioned in the introduction, soil uptake tends to increase  $\delta D$  while soil emission tends to decrease  $\delta D$  of  $H_2$ . The continuous decrease of  $\delta D$  with time in all Cabauw experiments and the eventual decrease of  $\delta D$  in all Speuld experiments clearly show that there is concurrent soil emission even with net uptake. Thus, the equilibrium  $H_2$  concentration in our experiments is not just a threshold concentration where microbial uptake stops, but the isotopic evolution shows that there is an active overlapping emission (Conrad, 1994).

### **3.2 Emission and uptake strength of $H_2$**

The production rate  $P = P_{\text{true}}$  and uptake rate constant  $k = k_{\text{true}}$  were obtained by applying exponential fits to the temporal evolution of  $H_2$ , and applying the corrections derived from the flask sampling model (appendix A) to the  $P_{\text{app}}$  and  $k_{\text{app}}$  obtained from the exponential fits (Fig. 6). The deposition velocity ( $v_d$ ),

455 production flux ( $F_p$ ), initial uptake flux ( $F_u$ ) and net flux at the beginning of the  
456 experiment ( $F_n$ ) were then calculated as follows:

457

$$v_d = kh \quad (7)$$

458

$$F_p = \frac{Ph}{V_M} \quad (8)$$

459

$$F_u = \frac{kc_1h}{V_M} \quad (9)$$

460

$$F_n = F_p - F_u \quad (10)$$

461

462 where  $h$ ,  $V_M$  and  $c_1$  are the chamber height, standard molar volume (=22.4 L  
463 mol<sup>-1</sup>) and H<sub>2</sub> mole fraction of the first flask, respectively. We note that with our  
464 method we derive  $v_d$  as deposition velocity for the gross uptake, unlike most of  
465 the results reported in the literature that just measured net uptake.

466

467 The strongest soil uptake occurs in the Speuld experiments (Table 1a), with a  
468 mean  $v_d$  of (0.17±0.02) (2 SE, n=12) cm s<sup>-1</sup> (SE represents standard error). On  
469 average, the Cabauw experiments show weaker soil uptake, with a mean  $v_d$  of  
470 (0.13±0.06) (2 SE, n=8) cm s<sup>-1</sup> for the net-uptake experiments (Table 1b) and

(0.06±0.03) (2 SE, n=9) cm s<sup>-1</sup> for the net-emission experiments (Table 2). In terms of the net H<sub>2</sub> flux  $F_n$ , this is (-26.5±4.8) (2 SE, n=12) nmol m<sup>-2</sup> s<sup>-1</sup> for Speuld experiments (Table 1a), (-13.6±8.6) (2 SE, n=8) nmol m<sup>-2</sup> s<sup>-1</sup> for Cabauw net-uptake experiments (Table 1b) and (49.5±29.8) (2 SE, n=9) nmol m<sup>-2</sup> s<sup>-1</sup> for Cabauw net-emission experiments (Table 2), indicating strong uptake, weaker uptake and strong emission of H<sub>2</sub>, respectively.

### 3.3 Fractionation during soil uptake

Soil uptake and soil emission have opposite effects on the isotopic composition of H<sub>2</sub> and can partly cancel each other. This will lead to additional uncertainty and we expect to obtain the most robust fractionation factor for soil uptake when the soil uptake is larger than the soil emission (Table 1a&b).

The resulting  $\alpha_{\text{soil}}$  for Speuld (Table 1a) varies from 0.913 to 0.955, with a mean value of 0.937±0.008 (2 SE, n=12). Error estimates for HH and HD mole fraction at time t and at equilibrium are considered for the final error estimates of  $\alpha_{\text{soil}}$  for each experiment.

Table 1b shows  $\alpha_{\text{soil}}$  of the Cabauw net-uptake experiments. It should be noted that the soil emitted  $\text{H}_2$  interferes much more with the fractionation during uptake in these Cabauw net-uptake experiments than for the Speuld experiments, which is illustrated by the consistent decrease in  $\delta\text{D}$  in the middle panel of Fig. 6. The derived values for  $\alpha_{\text{soil}}$  vary from 0.911 to 1.019 with a mean value of  $0.951 \pm 0.026$  (2 SE,  $n=8$ ) for these 8 selected Cabauw net-uptake experiments. Both the mean and the standard error are higher than for the Speuld experiments ( $0.937 \pm 0.008$ ), but the difference is not significant at the 0.1 confidence level.

To graphically illustrate the calculation of  $\alpha_{\text{soil}}$  with the mass balance model, we plot  $\ln \frac{c' - c'_{\text{e,app}}}{c'_1 - c'_{\text{e,app}}}$  versus  $\ln \frac{c - c_{\text{e,app}}}{c_1 - c_{\text{e,app}}}$  for all Speuld and Cabauw net-uptake experiments in Fig. 7. A linear fit is applied to all the data and the overall  $\alpha_{\text{soil,app}}$  is found to be  $0.947 \pm 0.004$  (95% CI). Applying a correction factor is not straightforward now because this analysis combines the results from different experiments. If we use the average of  $\alpha_{\text{soil,true}} / \alpha_{\text{soil,app}}$  ratios (0.998) for all net-uptake experiments in Table 3 as the correction coefficient for this overall  $\alpha_{\text{soil,app}}$ , the overall  $\alpha_{\text{soil}}$  is  $0.945 \pm 0.004$  (95% CI).

Fig. 8 shows  $\alpha_{\text{soil}}$  as a function of  $v_d$  for all Speuld experiments and Cabauw net-uptake experiments. The  $R^2$  value is nearly zero and the p-value is 0.53 for the linear regression of all experiments, so no significant correlation between  $\alpha_{\text{soil}}$

and  $v_d$  is found. Also, no significant correlation is found when considering the Speuld and Cabauw net-uptake experiments separately.

### 3.4 Isotopic signature of H<sub>2</sub> emitted from soil

As discussed in Section 2.4, the isotopic signature of H<sub>2</sub> emitted from the soil ( $\delta D_{\text{soil}}$ ) can be obtained from the mass balance model. In order to minimize the influence of soil uptake on the computed  $\delta D_{\text{soil}}$  and obtain the most robust result, we only consider the Cabauw experiments with strong soil emission and weak soil uptake ( $c_{e,\text{app}} > 1500$  ppb). In total, 9 Cabauw experiments are selected (Table 2) and a linear fit is applied to the plot of  $c'_{e,\text{app}} \ln \frac{c' - c'_{e,\text{app}}}{c'_1 - c'_{e,\text{app}}}$  versus  $c_{e,\text{app}} \ln \frac{c - c_{e,\text{app}}}{c_1 - c_{e,\text{app}}}$  for each experiment (Fig. 9). It can be seen that the linear function fits the data very well for each experiment. The slope of the linear fit yields  $P'_{\text{app}}/P_{\text{app}}$ . This  $P'_{\text{app}}/P_{\text{app}}$  ratio is used to calculate  $\delta D_{\text{soil,app}}$  (Eq. (5)). After correcting for the flask sampling effects (see Appendix A), the corresponding  $\delta D_{\text{soil}}$  values are shown in Table 2. The  $\delta D_{\text{soil}}$  value ranges from -629 ‰ to -451 ‰, with a mean value of  $(-530 \pm 40)$  ‰ (2 SE, n=9), which is very D-depleted, but still considerably enriched relative to the value around -700 ‰ expected for thermodynamic equilibrium between H<sub>2</sub> and H<sub>2</sub>O (Bottinga, 1969).

## 4. Discussion

### 4.1 Emission and uptake strength of H<sub>2</sub>

The deposition velocity  $v_d$  is a measure of the strength of soil uptake. Both microbial removal and diffusion can affect  $v_d$ , and they can both be influenced by the temperature and moisture content of the soil (Ehhalt and Rohrer, 2013a; 2013b). On average, the  $v_d$  obtained in this study is larger in the forest region (Table 1a) than in the grass/clover region (Table 1b and 2), in agreement with the conclusion from Ehhalt and Rohrer (2009).

The  $v_d$  of  $(0.06 \pm 0.03)$  cm s<sup>-1</sup> found in our Cabauw net-emission experiments (Table 2) is similar to those reported in Conrad and Seiler (1980) (0.07 cm s<sup>-1</sup>, both grass and clover) and Gerst and Quay (2001) (0.04 cm s<sup>-1</sup>, grass), while the  $v_d$  of  $(0.13 \pm 0.06)$  cm s<sup>-1</sup> in Cabauw net-uptake experiments (Table 1b) is larger than those studies with similar soil cover but close to values of 0.12 to 0.14 cm s<sup>-1</sup> found in savanna soil (Conrad and Seiler, 1985). The stronger soil uptake in Speuld forest ( $(0.17 \pm 0.02)$  cm s<sup>-1</sup>) agrees well with the beech forest results (0.06 to 0.22 cm s<sup>-1</sup>) in Förstel (1988) and Förstel and Führ (1992). However, other

studies at forest sites cited in Ehhalt and Rohrer (2009) showed lower  $v_d$  than our Speuld results. We note here that the  $v_d$  values reported in Conrad and Seiler (1980; 1985) were gross deposition velocities while those reported in Gerst and Quay (2001) were net deposition velocities. The specific method used to obtain  $v_d$  was not documented in the other studies.  $v_d$  obtained from our experiments are gross deposition velocities.

The net uptake flux  $F_n$  in our Speuld experiments and Cabauw net-uptake experiments is much larger than those found in Smith-Downey et al. (2008). They found a  $F_n$  of about  $-8 \text{ nmol m}^{-2} \text{ s}^{-1}$  for the forest, desert, and marsh, which was similar to that for loess loamy soil in Schmitt et al. (2009). Our results are within the  $F_n$  range found in the mixed wood plains by Constant et al. (2008b) and the Harvard forest by Meredith (2012). Previously at our Cabauw site, Popa et al. (2011) obtained a  $F_n$  of only  $-3 \text{ nmol m}^{-2} \text{ s}^{-1}$  by using the radon tracer method. However, the Cabauw net-uptake experiments used for this evaluation were from selected places where uptake was strong, while the results in Popa et al. (2011) represented the overall uptake in the footprint of the Cabauw site, which is a much larger area (tens of  $\text{km}^2$ ).

Khdhiri et al. (2015) performed microbiological analyses on soil samples from the Cabauw and Speuld sites, in order to find the drivers of soil  $\text{H}_2$  uptake. They observed that the  $\text{H}_2$  uptake rate under standard incubation conditions was



significantly lower for the Cabauw soil samples than for the Speuld ones, which is consistent with our findings. The main factors that explained the differences were the relative abundance of high affinity  $H_2$ -oxygenizing bacteria and the soil carbon content, both lower on average for the Cabauw site.

The emission of  $H_2$  from the soil is large for the Cabauw net-emission experiments, with  $F_n$  ranging from 13.7 to 150.2  $nmol\ m^{-2}\ s^{-1}$  and a median value of 41.0  $nmol\ m^{-2}\ s^{-1}$  (Table 2). One experiment, “CBW-28”, shows unusually high emission, with  $H_2$  increasing to 3010 ppb within 30 minutes. In comparison, Conrad and Seiler (1980) found a  $F_n$  of 23-32  $nmol\ m^{-2}\ s^{-1}$  for a clover field. Except for the experiments “CBW-28” and “CBW-31”, our Cabauw net-emission experiments are close to the  $F_n$  found by them. The variability in  $F_n$  could be attributed to different  $N_2$  fixation flux in our experiments, which could be affected by both spatial density of  $N_2$  fixation organisms and their  $N_2$  fixation activities. The  $N_2$  fixation activity could be regulated by various factors including temperature, moisture, light availability and carbon storage etc. (Belnap, 2001), which were not measured are therefore not discussed here.

## 4.2 Fractionation during soil uptake

596

597 Fractionation during soil uptake of  $H_2$  can happen during the diffusion into the  
598 soil and due to microbial removal within the soil. To further investigate the  
599 factors determining  $\alpha_{\text{soil}}$ , information about the soil cover is provided in Table  
600 1a&b. It is evident that no large differences exist between the Douglas fir,  
601 spruce and beech sites, i.e. the variability between sites is similar to the  
602 variability within sites. The small number of experiments impedes examining  
603 the possible small differences between sites. In order to investigate the diffusion  
604 effect, we removed the soil cover in experiments “SPU-8” and “SPU-12” at the  
605 same place of experiments “SPU-7” and “SPU-11”. The removal of leaves  
606 (“SPU-8”) and needles (“SPU-12”) increased  $\alpha_{\text{soil}}$  by  $\approx 0.014$ , thus towards  
607 smaller fractionation, which indicates that diffusion contributes to the  
608 fractionation. As  $v_d$  also increases when the soil cover is removed, faster  
609 deposition is associated with smaller fractionations in these experiments, which  
610 is similar to the results from Rice et al. (2011).

611

612 The  $\alpha_{\text{soil}}$  for the Cabauw net-uptake experiments is higher and more scattered  
613 than that for the Speuld experiments ( $0.951 \pm 0.026$  vs.  $0.937 \pm 0.008$ ). This could  
614 be caused by the interference of D-depleted  $H_2$  from the strong soil emission in  
615 Cabauw, which may not be perfectly captured via the mathematical models  
616 applied. As can be seen from the strong decline of  $\delta D$  with time in the middle  
617 panel of Fig. 6, though soil uptake of  $H_2$  dominates for the Cabauw net-uptake

experiments, soil production is still considerable. If part of the source signature is not taken into account properly and appears in  $\alpha_{\text{soil}}$ , then  $\alpha_{\text{soil}}$  will be larger, because soil production tends to decrease  $\delta D$  of  $H_2$ . This could explain why  $\alpha_{\text{soil}}$  is even larger than 1 in “CBW-7”.

The overall  $\alpha_{\text{soil}}$  (0.945) obtained by plotting  $\ln \frac{c' - c'_{e,\text{app}}}{c'_1 - c'_{e,\text{app}}}$  versus  $\ln \frac{c - c_{e,\text{app}}}{c_1 - c_{e,\text{app}}}$  and applying the average correction factor for all the Speuld and Cabauw net-uptake experiments is similar to the results of  $0.943 \pm 0.024$  from Gerst and Quay (2001) and  $0.94 \pm 0.01$  from Rahn et al. (2002a). They suggested that the overall  $\alpha_{\text{soil}}$  is more accurate as it is less susceptible to outliers. We argue here that the average  $\alpha_{\text{soil}}$  of all individual experiments in Speuld (0.937) and Cabauw (0.951) is representative for a spatially averaged fractionation factor for those sites and is useful for e.g. characterizing the phenomenon and comparing with other fractionation results. If all experiments are included in one fit, their weight for determining the slopes depends on how much  $H_2$  has been removed, so experiments with a lower  $c_{e,\text{app}}$  have a larger weight than experiments with a higher  $c_{e,\text{app}}$  (i.e. experiments with a higher  $v_d$  have a larger weight than experiments with a lower  $v_d$ ). The fractionation factor obtained by fitting all data together is therefore representative for a flux weighted average, which is the relevant number for the global atmospheric isotope budget.

### 4.3 Relationship between $\alpha_{\text{soil}}$ and $v_d$

Rice et al. (2011) proposed a significant positive correlation between  $\alpha$  and deposition velocity  $v_d$  in their soil uptake experiments. Fig. 8 shows that no significant correlation between  $\alpha_{\text{soil}}$  and  $v_d$  is found when considering all Speuld and Cabauw net-uptake experiments. The uptake rate is much stronger in the Speuld experiments ( $v_d \approx 0.17 \text{ cm s}^{-1}$ ) than in the study of Rice et al. (2011) ( $v_d \approx 0.04 \text{ cm s}^{-1}$ ), but the  $\alpha_{\text{soil}}$  is virtually identical (0.937 *versus* 0.934). Therefore, when the results from both studies are combined, the correlation reported in Rice et al. (2011) between  $\alpha_{\text{soil}}$  and  $v_d$  disappears. We suggest that a positive correlation between  $\alpha_{\text{soil}}$  and  $v_d$  may exist for a specific site where microbial species are similar. This was suggested from the simultaneous increase of both  $\alpha_{\text{soil}}$  and  $v_d$  in two experiments (“SPU-8” and “SPU-12”), when soil cover was removed at the same sampling location, as mentioned in Section 4.2.

We conclude that there is certainly not one single correlation between  $\alpha_{\text{soil}}$  and  $v_d$  that holds globally and the soil type might play an important role. Measurements at more sites may be needed to positively confirm whether local positive correlations between  $\alpha_{\text{soil}}$  and  $v_d$  are common.

#### 4.4 $\delta D$ of $H_2$ emitted from the soil

The present study is the first field study to report  $\delta D$  of  $H_2$  emitted from soils. The  $\delta D_{\text{soil}}$  values (-629 ‰ to -451 ‰) shown in Table 2 are less depleted than the  $H_2$  in isotopic equilibrium with water ( $\approx -700$  ‰). Previous observations from environmental  $H_2$  production yielded a  $\delta D$  of -628 ‰ for two seawater samples (Rice et al., 2010), -778 ‰ for a termite headspace sample and -690 ‰ for two headspace samples from a eutrophic water pond (Rahn et al., 2002b). Kawagucci et al. (2015) proposed that microbiological  $H_2$  consumption and production could destroy the thermal isotopic equilibrium between  $H_2$  and  $H_2O$  in low-temperature hydrothermal fluids. Luo et al. (1991) and Walter et al. (2012) found fractionation factors of 0.448, 0.401 and 0.363 for  $H_2$  generated from water by different  $N_2$ -fixing bacteria in the laboratory.

In order to compare our  $\delta D_{\text{soil}}$  with the fractionation factors between  $H_2$  and  $H_2O$  found by Luo et al. (1991) and Walter et al. (2012), we converted their fractionation factors to  $\delta D(H_2)$  by assuming the  $\delta D(H_2O)$  to be the same as that of global rainwater (-37.8 ‰, Hoffmann et al., 1998). This results in  $\delta D(H_2)$  values of -651 ‰ to -569 ‰ for their  $N_2$ -fixing bacteria. Although the ranges are considerable, it appears that the mean  $\delta D_{\text{soil}}$  (-530 ‰) obtained in our field

680 study is even higher than what was found for nitrogenase-derived H<sub>2</sub> in  
681 laboratory experiments.

682

683 It is known that H<sub>2</sub> produced by biogenic N<sub>2</sub> fixation can be largely recycled  
684 within the soil before entering the atmosphere (Evans et al., 1987; Conrad and  
685 Seiler, 1979; 1980). If this uptake process within the soil tends to increase the  
686  $\delta D$  of the remaining H<sub>2</sub>, as the soil uptake process for atmospheric H<sub>2</sub> does, then  
687 the H<sub>2</sub> entering the atmosphere will be less D-depleted than pure biogenic H<sub>2</sub>.  
688 However, if the fractionation factor of removal in the soil is similar to that  
689 determined from the net-uptake experiments ( $\approx 0.94$ ), a large fraction ( $f_{in}$ ) of H<sub>2</sub>  
690 needs to be removed in the soil before release to explain the D-enriched  $\delta D_{soil}$   
691 compared to the values reported in the literature. The fraction  $f_{in}$  could in  
692 principle be estimated from the Rayleigh equation:

$$(1 - f_{in})^{\alpha_{in}-1} = \frac{\delta D_{soil} + 1}{\delta D_0 + 1}$$

693 where  $\alpha_{in}$  is the fractionation constant of H<sub>2</sub> within soil,  $\delta D_0$  is the  $\delta D$  value of  
694 initial H<sub>2</sub> produced by N<sub>2</sub>-fixers, and  $\delta D_{soil}$  is the  $\delta D$  value of remaining H<sub>2</sub>  
695 emitted from soil that is measured in our experiments. By assuming  $\alpha_{in}=0.945$   
696 (overall fractionation factor as determined in our deposition experiments),  
697  $\delta D_{soil}=-530\text{‰}$  (averaged  $\delta D_{soil}$  of Cabauw net-emission experiments) and  $\delta D_0=-$   
698  $611\text{‰}$  (averaged of  $\delta D(H_2)$  derived from laboratory experiments in Luo et al.  
699 (1991) and Walter et al. (2012)), we would obtain  $f_{in}=0.97$ . That is, 97% of H<sub>2</sub>

produced by  $N_2$  fixation would be removed within soil before entering atmosphere. This is higher than the estimate from Conrad and Seiler (1979), which was from 30% to 90%. It should be noted that the estimation of  $f_{in}$  is very uncertain due to the lack of information about  $\alpha_{in}$  and  $\delta D_0$ . By using the lower limit of  $\alpha_{in}$  (0.911) in our experiment and the upper limit of  $\delta D_0$  (-569 ‰) in Luo et al. (1991) and Walter et al. (2012), we calculate a lower limit of  $f_{in}$  to be 0.62. The upper limit of  $f_{in}$  is 1.00 when  $\alpha_{in}$  approaches 1. For these calculations we have used a  $\delta D_{soil}$  of -530 ‰, but it varies from -629 ‰ to -451 ‰ in our experiments. We cannot rule out cases with  $\delta D_{soil} = \delta D_0$ , which yields a  $f_{in}$  of 0.

The deuterium enrichment in the emitted  $H_2$ , compared to the value expected in isotopic equilibrium with water, could also be caused by different fractionations induced by different enzymes and/or a potentially enriched deuterium content of the substrate water available for  $H_2$  production in Cabauw.  $H_2$  is generated from the reduction of hydrogen ions ( $H^+$  or  $D^+$ ) in intracellular water (Yang et al., 2012). It was found that the isotopic composition of intracellular water can be different from that of extracellular water due to metabolic processing (Kreuzer-Martin et al., 2006). Due to the differences in H-bonding and hydrogen ion transport, the fractionation may be different for different microbe species, which could result in different isotopic signatures of the produced  $H_2$ . Measurements of the isotopic composition of produced  $H_2$  may be a tool to investigate such effects.

722

723 Finally, we note that if our Cabauw net-emission experiments are analyzed with  
724 a simple Keeling plot approach (i.e. without considering uptake), the y axis  
725 intercept is -703 ‰. We know from the temporal evolution of H<sub>2</sub>, HD and δD  
726 that this model is not adequate and that uptake was significant in our  
727 experiments, so a simple Keeling plot analysis can be misleading if uptake is  
728 not considered.

729

730

## 731 **5. Conclusions**

732

733 This study investigated the isotope effects associated with the production and  
734 uptake of atmospheric H<sub>2</sub> by soil. Our aim was to quantify the fractionation  
735 factor  $\alpha_{\text{soil}}$  for H<sub>2</sub> deposition and the isotopic signature of H<sub>2</sub> emitted from the  
736 soil (δD<sub>soil</sub>) from experiments carried out at Speuld and Cabauw.

737

738 The experiments covered a wide range of conditions from situations with very  
739 strong net H<sub>2</sub> uptake to situations with very strong net H<sub>2</sub> emission. The  
740 superposition of deposition and production made the analysis with simple  
741 models like Rayleigh plot and Keeling plot impossible. Therefore, the mass  
742 balance model suggested by Rice et al. (2011) was used for evaluation.



743

744 The deposition velocity  $v_d$  was largest in the Speuld experiments ( $(0.17 \pm 0.02)$   
745  $\text{cm s}^{-1}$ ) where also the strongest net soil uptake occurred, followed by the  
746 Cabauw net-uptake experiments ( $(0.13 \pm 0.06) \text{ cm s}^{-1}$ ) and Cabauw net-emission  
747 experiments ( $(0.06 \pm 0.03) \text{ cm s}^{-1}$ ). The net  $\text{H}_2$  flux  $F_n$  was  $(-26.5 \pm 4.8) \text{ nmol m}^{-2}$   
748  $\text{s}^{-1}$  for Speuld experiments,  $(-13.6 \pm 8.6) \text{ nmol m}^{-2} \text{ s}^{-1}$  for Cabauw net-uptake  
749 experiments and  $(49.5 \pm 29.8) \text{ nmol m}^{-2} \text{ s}^{-1}$  for Cabauw net-emission  
750 experiments.

751

752 The mean fractionation factors  $\alpha_{\text{soil}}$  are  $0.937 \pm 0.008$  for the Speuld forest soil  
753 experiments and  $0.951 \pm 0.026$  for the Cabauw grassland experiments, which are  
754 representative for a spatial average and useful for comparisons with other  
755 fractionation studies. The Cabauw results may be affected by the relatively  
756 strong concomitant soil emissions. The overall  $\alpha_{\text{soil}}$  by considering all net-  
757 uptake experiments is  $0.945 \pm 0.004$ , which is representative for a flux weighted  
758 average and useful for global isotope budget estimates. The fractionation factors  
759 found in this work are in good agreement with previous studies.

760

761 No significant correlation between  $\alpha_{\text{soil}}$  and deposition velocity  $v_d$  was found  
762 while considering all of our experiments. The  $v_d$  were overall much larger in our  
763 study than those in Rice et al. (2011) and we obtained similar values for  $\alpha_{\text{soil}}$ .  
764 This demonstrates that the positive correlation that was found previously does

765 not hold globally. From two of our Speuld experiments,  $\alpha_{\text{soil}}$  increased after the  
766 removal of leaves or needles above the soil. This indicates that there may be a  
767 fractionation associated with diffusion through the surface layer of leaves or  
768 needles during soil uptake, but more experiments are required to confirm this.

769

770 The isotopic analysis clearly showed that the net uptake was always a  
771 superposition of a larger gross uptake and a gross emission flux. In Cabauw, the  
772 emission strength was very large at locations where clover was present. Using a  
773 simple mass balance approach, the isotopic composition of the emitted  $\text{H}_2$  was  
774 determined to be  $(-530 \pm 40) \text{‰}$ , which is significantly higher than the value  
775 expected for  $\text{H}_2\text{O} - \text{H}_2$  isotope equilibrium. Although limited, other published  
776 data on  $\text{H}_2$  produced biologically via nitrogenase show also a tendency to more  
777 enriched values. An additional isotope enrichment in our field soil study could  
778 originate from fractionation during the recycling of  $\text{H}_2$  within the soil before it  
779 enters the atmosphere.

780

## Appendix A

### A1. Flask sampling model

A mathematical model is used to simulate the sampling and to correct for the effects of the flask sampling method on the values of uptake rate constant ( $k$ ), production rate ( $P$ ), fractionation factor ( $\alpha_{\text{soil}}$ ) and isotopic signature of  $\text{H}_2$  produced from soil ( $\delta\text{D}_{\text{soil}}$ ). We start with a pair of known (*true*) uptake and production rates and simulate the evolution of the mole fractions of  $\text{H}_2$  and HD in the flasks and chamber. From the modeled mole fractions we calculate the *apparent* uptake and production rates and derive the correction needed to obtain the *true* uptake and production rates from measurement of the *apparent* rates in actual experiments.

#### A1.1 Mathematical description of the flask sampling model

The sampling setup is shown in Fig. 2 of the main paper. After 10 minutes of flushing, the chamber and the flasks contain ambient air with the prevailing  $\text{H}_2$  and HD mole fractions. In the following we denote  $c_1(t)$ ,  $c_2(t)$ ,  $c_3(t)$ ,  $c_4(t)$  and  $c_0(t)$  the  $\text{H}_2$  mole fractions for the first, second, third, forth flask and the chamber, respectively. The moment when the first flask and the chamber lid are

802 closed is considered the starting time of the experiment ( $t=0$ ). From this point  
 803 on, only the chamber, the second, third and fourth flask are connected, and the  
 804 initial  $H_2$  mole fraction inside them is  $c_0(0) = c_2(0) = c_3(0) = c_4(0) = c_1$ . We start  
 805 a simulation with an input uptake rate constant ( $k_{\text{true}}$ ) and an input production  
 806 rate ( $P_{\text{true}}$ ). The simulation of the flask sampling is based on Eqs. (A1)-(A4)  
 807 shown below.

808

809 Assuming that the air in each flask and in the chamber is well-mixed during the  
 810 entire sampling process, the time evolution for the second flask  $c_2(t)$ , the third  
 811 flask  $c_3(t)$ , the forth flask  $c_4(t)$  and the chamber  $c_0(t)$  in the first 10 minutes after  
 812 starting the experiment can be expressed as:

813

$$\frac{dc_2(t)}{dt} = \frac{f}{V} c_0(t) - \frac{f}{V} c_2(t) \quad (\text{A1})$$

814

$$\frac{dc_3(t)}{dt} = \frac{f}{V} c_2(t) - \frac{f}{V} c_3(t) \quad (\text{A2})$$

815

$$\frac{dc_4(t)}{dt} = \frac{f}{V} c_3(t) - \frac{f}{V} c_4(t) \quad (\text{A3})$$

816

$$\frac{dc_0(t)}{dt} = \frac{f}{V'} c_4(t) - \frac{f}{V'} c_0(t) + (P_{\text{true}} - k_{\text{true}} c_0(t)) \quad (\text{A4})$$

817

818 where  $V$  and  $V'$  are the air volumes of the flask and chamber, and  $f$  is the flow  
819 rate. These differential equations are solved using the Matlab ODE solvers at  
820 time steps of 0.01 min. The input parameters are  $c_0(0)$ ,  $P_{\text{true}}$ ,  $k_{\text{true}}$ ,  $V$ ,  $V'$  and  $f$ .  
821 For each time step the solvers calculate the hydrogen flux into and out of the  
822 chamber and each flask, as well as the new mole fractions there.

823

824 After 10 minutes, the second flask is closed and now contains air with mole  
825 fraction  $c_2 = c_2(10 \text{ min})$ . From this point on, only the chamber, the third and the  
826 fourth flask are connected, and the time evolution of the mole fractions can be  
827 expressed as:

828

$$\frac{dc_3(t)}{dt} = \frac{f}{V} c_0(t) - \frac{f}{V} c_3(t) \quad (\text{A5})$$

829

$$\frac{dc_4(t)}{dt} = \frac{f}{V} c_3(t) - \frac{f}{V} c_4(t) \quad (\text{A6})$$

830

$$\frac{dc_0(t)}{dt} = \frac{f}{V'} c_4(t) - \frac{f}{V'} c_0(t) + (P_{\text{true}} - k_{\text{true}} c_0(t)) \quad (\text{A7})$$

831

832 After another 10 minutes of sampling, the third flask is closed  $c_3 = c_3(20 \text{ min})$ ,  
833 and only the chamber and the fourth flask are connected. Then, the time  
834 evolution for the fourth flask and the chamber can be expressed as:

835

$$\frac{dc_4(t)}{dt} = \frac{f}{V} c_0(t) - \frac{f}{V} c_4(t) \quad (\text{A8})$$

836

$$\frac{dc_0(t)}{dt} = \frac{f}{V'} c_4(t) - \frac{f}{V'} c_0(t) + (P_{\text{true}} - k_{\text{true}} c_0(t)) \quad (\text{A9})$$

837

838 The H<sub>2</sub> mole fraction inside the chamber and the fourth flask at time t=30  
839 minutes is  $c_0(30)$  and  $c_4(30)$ .

840

841 In the end, a set of four flasks with mole fractions  $c_1(0)$ ,  $c_2(10 \text{ min})$ ,  $c_3(20 \text{ min})$   
842 and  $c_4(30 \text{ min})$  is obtained. By fitting this set of four data points with an  
843 exponential function  $c = ae^{-k_{\text{app}}t} + c_{\text{e,app}}$  (see Eq. (2) in the main paper), we  
844 can obtain the *apparent* soil uptake rate constant ( $k_{\text{app}}$ ) and equilibrium  
845 concentration ( $c_{\text{e,app}}$ ) and further calculate *apparent* production rate  
846 ( $P_{\text{app}} = k_{\text{app}} c_{\text{e,app}}$ ). These apparent rates  $k_{\text{app}}$  and  $P_{\text{app}}$  are different from the assumed  
847 *true* rates  $k_{\text{true}}$  and  $P_{\text{true}}$ . The flask sampling model enables us to establish a  
848 relation between  $k_{\text{app}}$  and  $P_{\text{app}}$  and  $k_{\text{true}}$  and  $P_{\text{true}}$ , so that  $k_{\text{true}}$  and  $P_{\text{true}}$  can be  
849 derived from  $k_{\text{app}}$  and  $P_{\text{app}}$  in actual experiments, where the true values are  
850 unknown. To accomplish this, simulations are carried out with a wide range of  
851 values for  $k_{\text{true}}$  and  $P_{\text{true}}$ , and a corresponding dataset of  $k_{\text{app}}$  and  $P_{\text{app}}$  is generated.

Similarly, we use a new set of input uptake rate constant  $k'_{\text{true}}$  and production rate  $P'_{\text{true}}$  for HD, and generate a corresponding dataset of  $k'_{\text{app}}$  and  $P'_{\text{app}}$ .

## **A1.2 The correction coefficients for $k$ and $P$**

Here we discuss an example of the relationship between  $k_{\text{true}}$  and  $k_{\text{app}}$  for the setup used in some Cabauw experiments ( $V=22.8$  L,  $f=2$  L min<sup>-1</sup> and  $\Delta t=10$  min). The pressure inside the flasks is 200 kPa and the pressure inside the chamber is 100 kPa. The relationship between  $k_{\text{true}}/k_{\text{app}}$  and  $k_{\text{app}}$  is shown in Fig. 10a. The ratio  $k_{\text{true}}/k_{\text{app}}$  varies between 1.45 to 1.61 for our  $k_{\text{app}}$  range of 0.04 to 0.30 min<sup>-1</sup>. This relationship does not depend on  $P_{\text{true}}$  (with  $P_{\text{true}}$  varying from 50 to 650 ppb min<sup>-1</sup>). An additional uncertainty can arise from incorrect timing of the flask sampling, but sampling times should be correct within few seconds, which may lead to an additional uncertainty of below 1%. The uncertainty of the flow rate obtained from the rotameter due to variations in ambient pressure and temperature that were not recorded is less than 4%, and the effect on the ratio  $k_{\text{true}}/k_{\text{app}}$  ratio is below 1%. We can retrieve  $k_{\text{true}}$  by multiplying  $k_{\text{app}}$  with the modeled value of  $k_{\text{true}}/k_{\text{app}}$  for each experiment. The ratio  $k_{\text{true}}/k_{\text{app}}$  for each experiment is shown in Table 3. It depends on experimental setup and  $k_{\text{app}}$  of each experiment, with a range of 1.177 to 1.589.

After retrieving  $k_{\text{true}}$  from  $k_{\text{app}}$ , we investigate the relationship between  $P_{\text{true}}/P_{\text{app}}$  and  $P_{\text{app}}$  for a fixed value of  $k_{\text{true}}$  (Fig. 10b). The ratio  $P_{\text{true}}/P_{\text{app}}$  depends slightly on  $P_{\text{app}}$  and  $k_{\text{true}}$ , ranging from 1.40 to 1.59 for a wide  $P_{\text{app}}$  range of 30 to 450 ppb  $\text{min}^{-1}$  and a wide  $k_{\text{true}}$  range of 0.05 to 0.45  $\text{min}^{-1}$ . As for the correction of  $k$ , uncertainties arising from incorrect timing of the flask sampling and from pressure and temperature variations and their effect on the flow rate lead to additional uncertainties of  $P_{\text{true}}/P_{\text{app}}$  ratio below 1%, which are not considered. We can retrieve  $P_{\text{true}}$  by multiplying  $P_{\text{app}}$  with  $P_{\text{true}}/P_{\text{app}}$  for each experiment after having determined  $k_{\text{true}}$  from  $k_{\text{app}}$ . The ratio  $P_{\text{true}}/P_{\text{app}}$  for each experiment is shown in Table 3 and depends on the experimental setup,  $P_{\text{app}}$  and  $k_{\text{app}}$  of each experiment. It ranges from 1.152 to 2.759 for most experiments, with an exception of 7.472 for experiment SPU-2 where a very small  $P_{\text{app}}$  of 0.67 ppb  $\text{min}^{-1}$  is found. Although the ratio  $P_{\text{true}}/P_{\text{app}}$  of experiment SPU-2 is high,  $P_{\text{true}}$  of SPU-2 is still smaller than the rest of the experiments.  $P_{\text{true}}/P_{\text{app}}$  ratios for experiments SPU-10 and SPU-11 are null because these two experiments show a  $P_{\text{app}}$  of zero.

### A1.3 The correction coefficients for $\alpha_{\text{soil}}$ and $\delta D_{\text{soil}}$

In our experiments, the uncertainties of  $k_{\text{app}}$  and  $k'_{\text{app}}$  derived from exponential fits to the time evolution of HH and HD are rather large, which results in a large scatter of  $\alpha_{\text{soil,app}}$  if  $\alpha_{\text{soil,app}}$  is calculated directly as  $k'_{\text{app}}/k_{\text{app}}$ . Thus, we obtained



895  $\alpha_{\text{soil,app}}$  by plotting  $\ln \frac{c' - c'_{\text{e,app}}}{c'_1 - c'_{\text{e,app}}}$  versus  $\ln \frac{c - c_{\text{e,app}}}{c_1 - c_{\text{e,app}}}$  (Fig. 7) for each experiment

896 which yields a smaller scatter for  $\alpha_{\text{soil,app}}$ .

897

898 Correction coefficients to convert  $\alpha_{\text{soil,app}}$  to  $\alpha_{\text{soil,true}}$  are obtained using the flask

899 sampling model by comparing  $\alpha_{\text{soil,true}}$  used as input for the model run to  $\alpha_{\text{soil,app}}$

900 derived from the plot of  $\ln \frac{c' - c'_{\text{e,app}}}{c'_1 - c'_{\text{e,app}}}$  versus  $\ln \frac{c - c_{\text{e,app}}}{c_1 - c_{\text{e,app}}}$  of the output values, like

901 in the experiments. Fig. 10c shows  $\alpha_{\text{soil,true}}/\alpha_{\text{soil,app}}$  as a function of  $\alpha_{\text{soil,app}}$  for a

902 wide  $\delta D_{\text{soil,true}}$  range of -750‰ to -250‰ with the sampling setup described

903 above ( $V'=22.8$  L,  $f=2$  L min<sup>-1</sup> and  $\Delta t=10$  min) for  $k_{\text{true}}=0.25$  min<sup>-1</sup> and  $P_{\text{true}}=50$

904 ppb min<sup>-1</sup>. In this case the correction factor  $\alpha_{\text{soil,true}}/\alpha_{\text{soil,app}}$  varies from 0.98 to

905 1.00 for a  $\alpha_{\text{soil,app}}$  range of 0.90 to 1.00, and it does not depend on  $\delta D_{\text{soil,true}}$ . Thus,

906 after retrieving  $k_{\text{true}}$  and  $P_{\text{true}}$  as described in Section A1.2, we can retrieve  $\alpha_{\text{soil,true}}$

907 from  $\alpha_{\text{soil,app}}$  for each experiment. The correction factors range from 0.984 to

908 1.007, depending on the experimental setup and  $\alpha_{\text{soil,app}}$  of each experiment

909 (Table 3).

910

911 Similarly, in our experiments, the uncertainties of  $P_{\text{app}}$  and  $P'_{\text{app}}$  derived from

912 exponential fits of time evolution of HH and HD are large, which results in a

913 large scatter of  $\delta D_{\text{soil,app}}$  if  $\delta D_{\text{soil,app}}$  is calculated directly from these  $P'_{\text{app}}$  and  $P_{\text{app}}$ .

914 We therefore obtained the ratio  $P'_{\text{app}}/P_{\text{app}}$  by plotting  $c'_{\text{e,app}} \ln \frac{c' - c'_{\text{e,app}}}{c'_1 - c'_{\text{e,app}}}$  versus

915  $c_{e,app} \ln \frac{c-c_{e,app}}{c_1-c_{e,app}}$  (Fig. 9) and calculated  $\delta D_{soil,app}$  from Eq. (4). This yielded  
 916 smaller scatter for  $\delta D_{soil,app}$ . After retrieving  $k_{true}$ ,  $P_{true}$  and  $\alpha_{soil,true}$  as described  
 917 above, we used the flask sampling model again to derived correction factors by  
 918 comparing  $\delta D_{soil,true}$  used as model input with  $\delta D_{soil,app}$  obtained from  
 919  $c'_{e,app} \ln \frac{c'-c'_{e,app}}{c'_1-c'_{e,app}}$  versus  $c_{e,app} \ln \frac{c-c_{e,app}}{c_1-c_{e,app}}$  of the model output, and retrieve  
 920  $\delta D_{soil,true}$  from  $\delta D_{soil,app}$  for each experiment. Fig. 10d shows  
 921  $(\delta D_{soil,true}+1)/(\delta D_{soil,app}+1)$  as a function of  $(\delta D_{soil,app}+1)$  for a  $\alpha_{soil,true}$  range of 0.90  
 922 to 1.00 with the sampling setup described above ( $V'=22.8$  L,  $f=2$  L min<sup>-1</sup> and  
 923  $\Delta t=10$  min) for  $k_{true}=0.25$  min<sup>-1</sup> and  $P_{true}=50$  ppb min<sup>-1</sup>. The ratio  
 924  $(\delta D_{soil,true}+1)/(\delta D_{soil,app}+1)$  changes from 0.99 to 1.05 for a wide  $(\delta D_{soil,app}+1)$   
 925 range of 0.25 to 0.65. It can be seen that the  $(\delta D_{soil,true}+1)/(\delta D_{soil,app}+1)$  ratio  
 926 depends slightly on  $\alpha_{soil,true}$  at a fixed  $(\delta D_{soil,app}+1)$ , with a maximum difference  
 927 of about 1% for a  $\alpha_{soil,true}$  range of 0.90 to 1.00. The ratio  
 928  $(\delta D_{soil,true}+1)/(\delta D_{soil,app}+1)$  for each net-emission experiment is shown in Table 3,  
 929 ranging from 1.007 to 1.048. The largest difference between  $\delta D_{soil,true}$  and  
 930  $\delta D_{soil,app}$  is 21‰ for CBW-8. The mean  $\delta D_{true}$  and  $\delta D_{app}$  for these net emission  
 931 experiments are -530‰ and -538‰, respectively.

932

933 In conclusion, the effect of the flask sampling process is relatively small for  $\alpha_{soil}$   
 934 and  $\delta D_{soil}$ , but considerable for the uptake rate constants  $k$  and  $k'$  and emission  
 935 rates  $P$  and  $P'$ . The flask sampling model allows to derive corresponding

corrections that have been applied to correct for the bias introduced by the flask sampling system.

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1246 **Tables**

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1248 Table 1. The deposition velocity ( $v_d$ ), fractionation factor ( $\alpha_{\text{soil}}$ ) as well as its error estimate,  
 1249 and soil cover information for each Speuld experiment (a) and Cabauw net-uptake  
 1250 experiment (b). The STDEV represents standard deviation and SE represents standard error.

1251 The errors of  $\alpha_{\text{soil}}$  represent the 95% confidence interval (CI) for  $\alpha_{\text{soil,app}}$  obtained from

1252  $\ln \frac{c' - c'_{\text{e,app}}}{c'_1 - c'_{\text{e,app}}}$  versus  $\ln \frac{c - c_{\text{e,app}}}{c_1 - c_{\text{e,app}}}$ .

(a)	$F_n$ (nmol m <sup>-2</sup> s <sup>-1</sup> )	$v_d$ (cm s <sup>-1</sup> )	$\alpha_{\text{soil}}$	Error $\alpha_{\text{soil}}$	soil cover
SPU-1	-30.1	0.20	0.924	0.032	D. fir, moss
SPU-2	-35.3	0.22	0.948	0.028	D. fir, needles
SPU-3	-37.7	0.20	0.945	0.008	D. fir, moss
SPU-4	-26.1	0.16	0.913	0.004	D. fir, moss
SPU-5	-24.9	0.16	0.918	0.006	D. fir, moss
SPU-6	-13.2	0.12	0.951	0.031	D. fir, moss
SPU-7	-19.6	0.12	0.939	0.005	beech, leaves
SPU-8	-28.4	0.16	0.955	0.008	<i>Same subsite as SPU-7, leaves removed</i>
SPU-9	-20.4	0.12	0.925	0.002	beech, leaves
SPU-10	-22.3	0.13	0.949	0.060	spruce, moss
SPU-11	-19.4	0.13	0.936	0.068	spruce, needles
SPU-12	-40.5	0.28	0.947	0.004	<i>Same subsite as SPU-11, needles removed</i>
MEAN	-26.5	0.17	0.937	/	/

STDEV	8.2	0.05	0.014	/	/
SE	2.4	0.01	0.004	/	/

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(b)	$F_n$ (nmol m <sup>-2</sup> s <sup>-1</sup> )	$v_d$ (cm s <sup>-1</sup> )	$\alpha_{\text{soil}}$	Error $\alpha_{\text{soil}}$	soil cover
CBW-5	-6.6	0.04	0.943	0.004	few clover, grass
CBW-7	-3.1	0.03	1.019	0.005	few clover, grass
CBW-16	-22.9	0.18	0.993	0.001	bare soil, few grass
CBW-18	-39.3	0.24	0.950	0.054	grass
CBW-19	-7.4	0.14	0.935	0.105	grass
CBW-20	-14.9	0.20	0.940	0.260	bare soil
CBW-25	-8.0	0.12	0.911	0.014	clover, grass
CBW-26	-6.1	0.09	0.916	0.038	grass
MEAN	-13.6	0.13	0.951	/	/
STDEV	12.2	0.08	0.037	/	/
SE	4.3	0.03	0.013	/	/

1255 Table 2. Net flux, deposition velocity and  $\delta D_{\text{soil}}$  (including error) obtained from the mass  
 1256 balance model for the net  $H_2$  emission experiments.

Net emission	$F_n$ (nmol m <sup>-2</sup> s <sup>-1</sup> )	$v_d$ (cm s <sup>-1</sup> )	$\delta D_{\text{soil}}$ (‰)	Error $\delta D_{\text{soil}}$ (‰)
CBW-8	24.5	0.05	-535	53
CBW-10	16.1	0.03	-460	17
CBW-14	13.7	0.02	-629	21
CBW-17	20.3	0.03	-542	1
CBW-21	42.0	0.04	-574	3
CBW-28	150.2	0.14	-488	83
CBW-30	41.0	0.05	-580	7
CBW-31	92.0	0.09	-509	7
CBW-33	46.2	0.10	-451	52
MEAN	49.5	0.06	-530	/
STDEV	44.7	0.04	59	/
SE	14.9	0.01	20	/

- 1 Table 3. Sampling information and the correction coefficients ( $k_{\text{true}}/k_{\text{app}}$ ,  $P_{\text{true}}/P_{\text{app}}$ ,  $\alpha_{\text{soil,true}}/\alpha_{\text{soil,app}}$  and  $(\delta D_{\text{soil,true}}+1)/(\delta D_{\text{soil,app}}+1)$  used
- 2 for each experiments. Size S refers to small chamber and size L refers to large chamber.

Exp.	Pressure (kPa)	Flow rate (L min <sup>-1</sup> )	Size	$\Delta t$ (min)	$k_{\text{app}}$ (min <sup>-1</sup> )	$P_{\text{app}}$ (ppb min <sup>-1</sup> )	$k_{\text{true}}/k_{\text{app}}$	$P_{\text{true}}/P_{\text{app}}$	$\alpha_{\text{soil,true}}/\alpha_{\text{soil,app}}$	$(\delta D_{\text{soil,true}}+1)/$ $(\delta D_{\text{soil,app}}+1)$
SPU-1	200	2	S	10	0.199	4.12	1.494	1.601	0.984	/
SPU-2	200	2.2	S	5	0.206	0.67	1.589	7.472	0.998	/
SPU-3	200	3.1	S	5	0.204	3.58	1.496	2.475	0.999	/
SPU-4	200	2.8	S	5	0.160	7.51	1.526	2.136	1.004	/
SPU-5	200	2.6	S	5	0.156	4.16	1.546	2.759	1.004	/
SPU-6	160	3.2	L	5	0.232	7.61	1.184	1.446	0.999	/
SPU-7	160	3.2	S	5	0.128	5.40	1.418	2.264	1.006	/
SPU-8	160	2.5	S	5	0.172	4.23	1.438	2.381	1.001	/
SPU-9	160	2.8	S	5	0.128	4.56	1.440	2.513	1.007	/
SPU-10	180	2.7	S	5	0.128	/	1.502	/	1.005	/
SPU-11	160	2.2	S	5	0.130	/	1.490	/	1.006	/
SPU-12	180	2.3	S	5	0.272	11.30	1.529	1.720	0.994	/
CBW-5	200	2	L	10	0.086	18.24	1.204	1.248	1.001	/
CBW-7	200	1.9	L	10	0.048	11.57	1.260	1.361	0.999	/
CBW-16	210	2.1	S	10	0.183	45.21	1.498	1.505	0.999	/
CBW-18	200	2	S	10	0.240	38.07	1.532	1.527	0.986	/
CBW-19	200	2	S	10	0.145	56.69	1.457	1.463	0.991	/
CBW-20	200	2	S	10	0.196	65.81	1.491	1.494	0.988	/
CBW-25	200	2	S	10	0.122	44.85	1.449	1.460	0.994	/
CBW-26	200	2	S	10	0.088	31.05	1.452	1.475	1.002	/
CBW-8	200	2	S	10	0.044	82.92	1.542	1.438	/	1.048
CBW-10	200	2.6	L	10	0.069	111.00	1.177	1.152	/	1.010
CBW-14	200	2.5	L	10	0.035	82.53	1.251	1.166	/	1.042
CBW-17	220	2.1	L	10	0.047	117.40	1.268	1.198	/	1.024

CBW-21	220	2	L	10	0.078	232.20	1.209	1.179	/	1.008
CBW-28	175	1.8	S	10	0.146	440.90	1.412	1.402	/	1.018
CBW-30	200	2	L	10	0.090	237.70	1.202	1.180	/	1.008
CBW-31	200	2	S	10	0.098	275.10	1.451	1.422	/	1.007
CBW-33	200	2	S	10	0.107	166.50	1.449	1.430	/	1.007

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## Figures

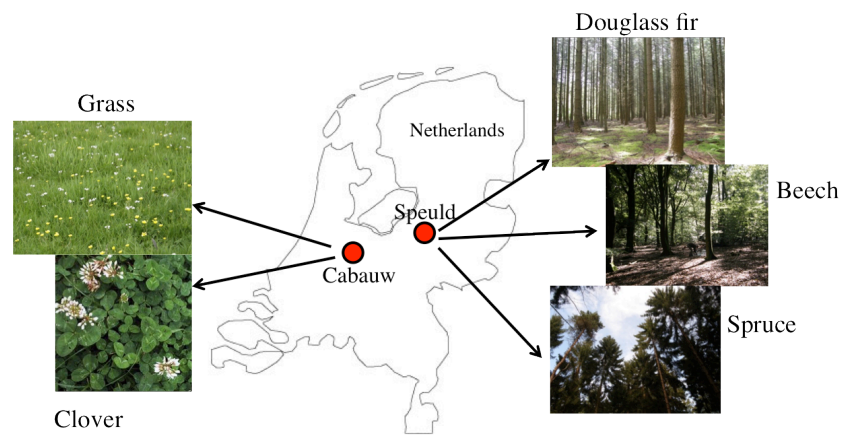


Fig. 1. The location of the two sampling sites (Cabauw and Speuld) in the Netherlands, as well as the plant species there.

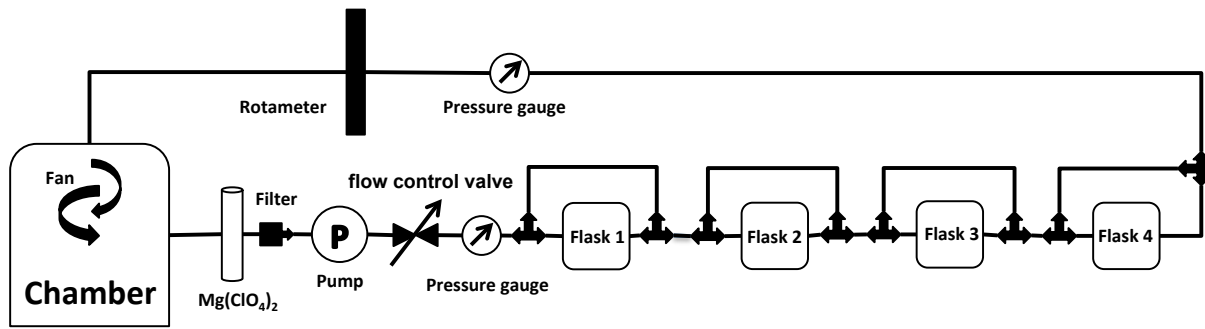


Fig. 2. Scheme of the sampling setup using the closed-cycle air sampler. The volume of the soil chamber was 22.8 L and the volume of each flask was 1 L.

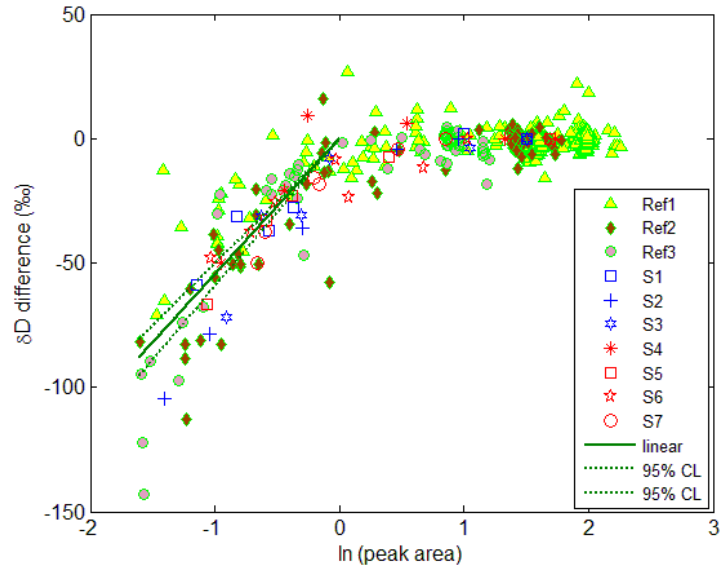


Fig. 3. Difference of  $\delta D$  from the assigned value for different gases including reference gases (Ref1-3) and laboratory flask samples (S1-7). A linear function ( $y = 54.6x$ ) was fit to the data with peak area between 0.2 and 1.0 V s (green solid line; the dashed lines represent the 95% confidence interval of the fit). This function was used to correct the soil experiment data that were measured at low peak areas.



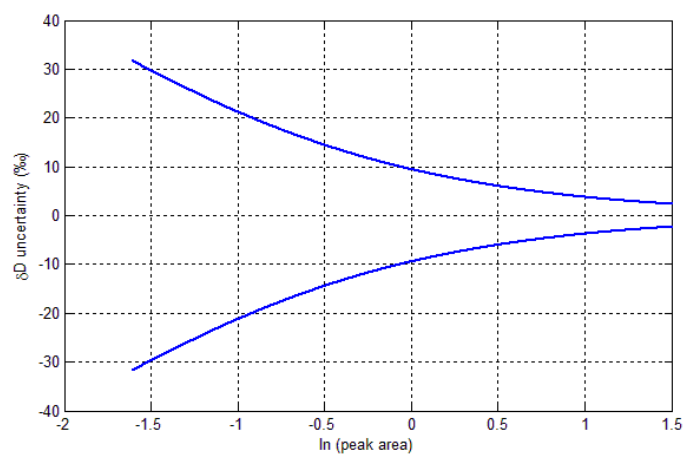


Fig. 4. Calculated total assigned uncertainty of  $\delta D$  (consisting of analytical uncertainty and uncertainty arising from the linearity correction) for air samples with  $\ln(\text{peak area})$  ranging from -1.6 to 1.5.

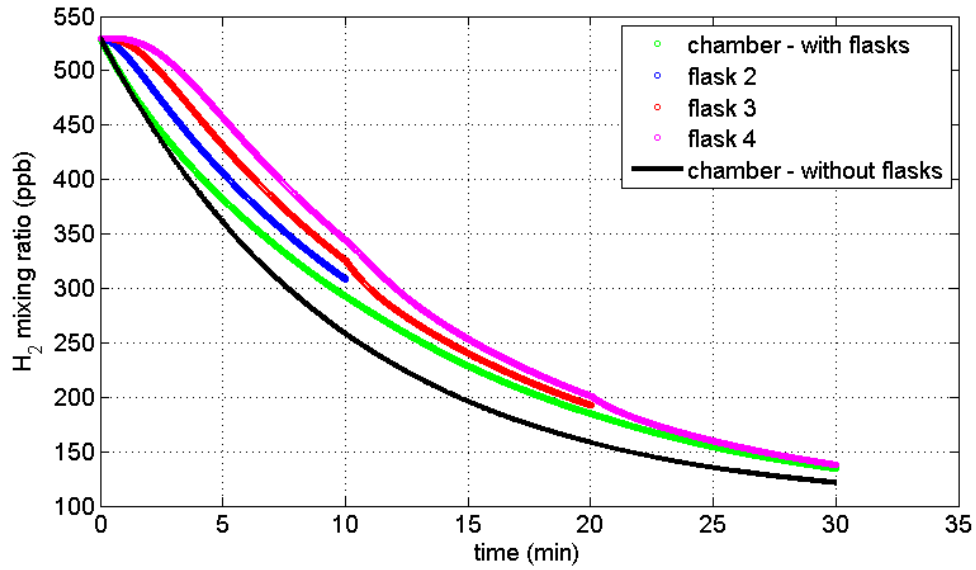


Fig. 5. Results of the flask sampling model with the following parameters:  $k=0.1 \text{ min}^{-1}$ ,  $P=10 \text{ ppb min}^{-1}$  and  $c_1(t=0)=530 \text{ ppb}$ . The figure shows the evolution of  $\text{H}_2$  mole fraction in the chamber (green curve), in flask 2 (blue curve), flask 3 (red curve) and flask 4 (magenta curve) as a function of time, and what would be expected for a chamber without flasks (black curve). Flask 1 was closed before closing the chamber (at time 0 when all volumes contained the same air).

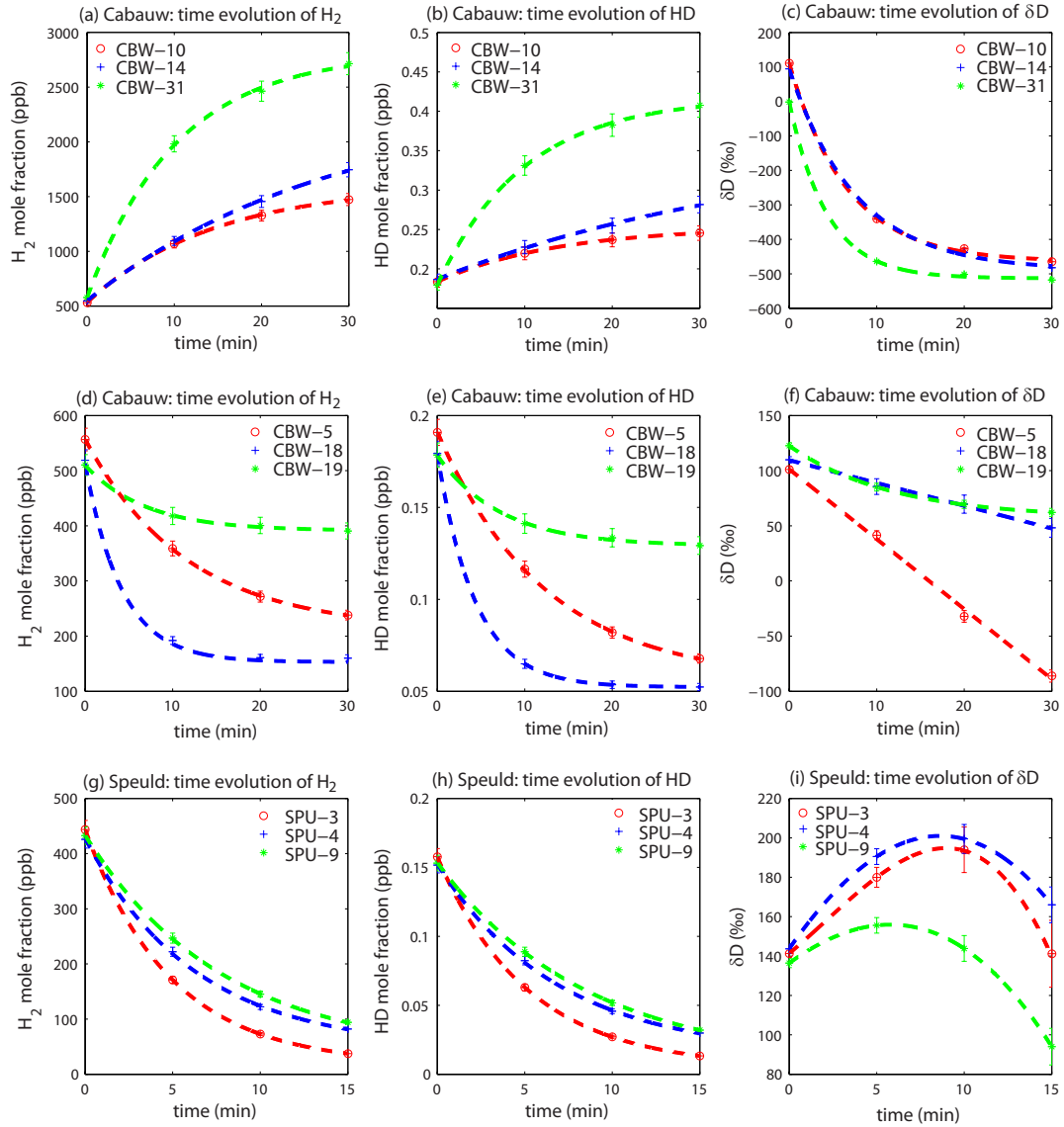


Fig. 6. Time evolution of  $H_2$ , HD and  $\delta D$  in Cabauw (upper and middle panels) and in Speuld (lower panel) for representative experiments. HD is calculated from  $H_2$  and  $\delta D$ . The  $H_2$  data are fitted with an exponential function of the form:  $c = (c_1 - c_{e,app})e^{-k_{app}t} + c_{e,app}$ , where  $c_1$  and  $c_{e,app}$  are the  $H_2$  mole fractions initially and in equilibrium, and  $k_{app}$  is the apparent soil uptake rate constant for  $H_2$ . A similar exponential function is applied to the HD data. Error estimates for  $H_2$ , HD and  $\delta D$  are shown. The connecting lines for  $\delta D$  data are included to guide the eye.

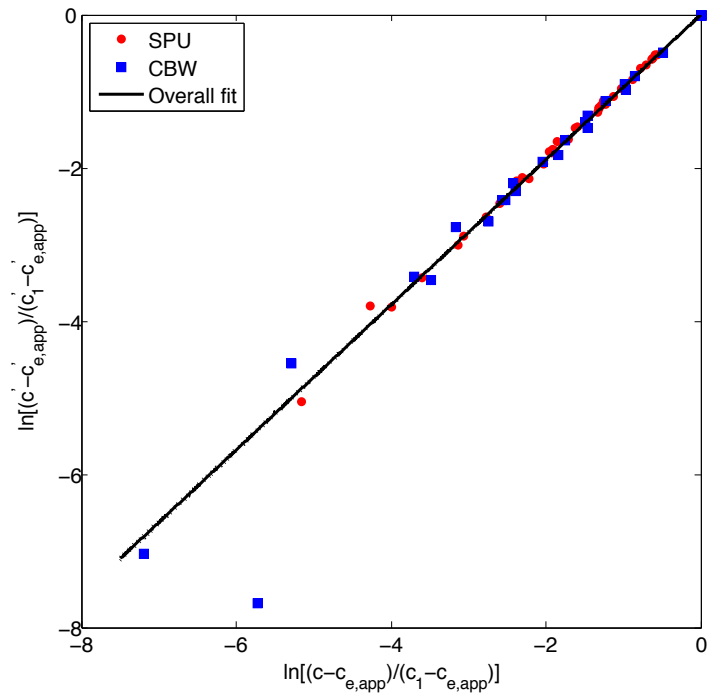


Fig. 7. Plot of  $\ln \frac{c' - c'_{e,app}}{c'_1 - c'_{e,app}}$  versus  $\ln \frac{c - c_{e,app}}{c_1 - c_{e,app}}$  for all Speuld and Cabauw net-uptake experiments. The slope of the linear fit to the data returns the fractionation factor  $\alpha_{soil,app} = 0.947 \pm 0.004$  (95% CI). Errors in x and y direction for each data point were considered. One outlier (“CBW-18”) was not included in the fitting. The 95% confidence intervals of the fit line are included as dashed lines but largely overlap with the fit line.

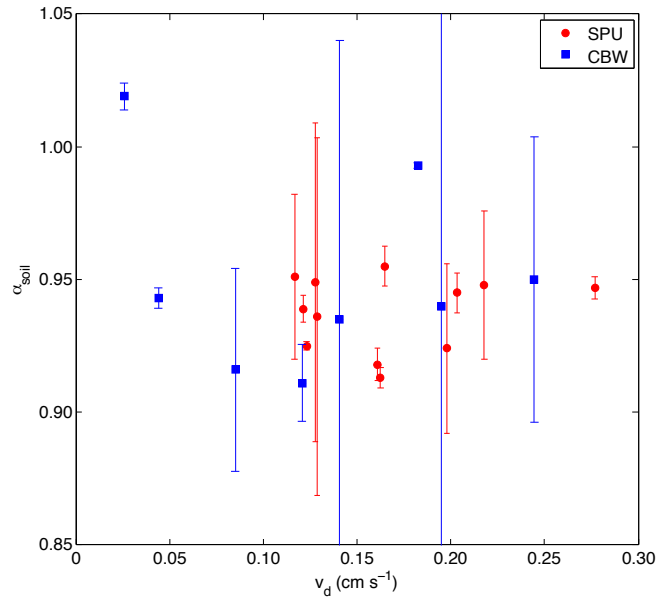
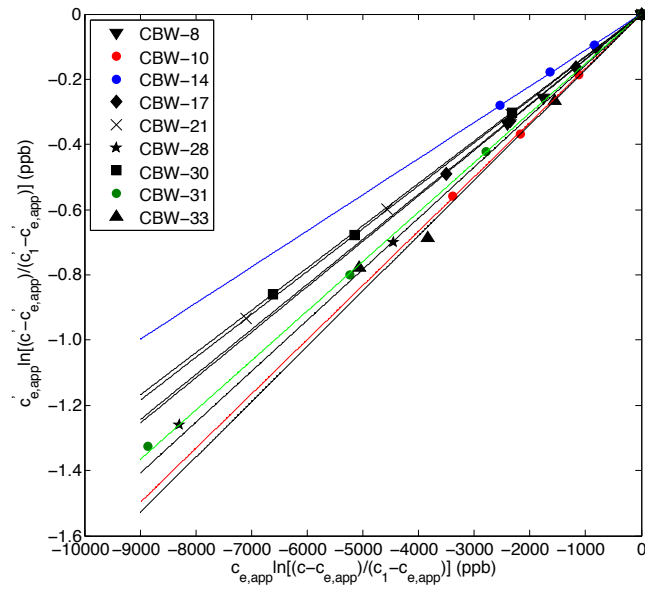


Fig. 8. Correlation between  $\alpha_{\text{soil}}$  and  $v_d$  for all Speuld experiments and Cabauw net-uptake experiments. The errors for  $\alpha_{\text{soil}}$  were taken from Table 1.

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1399 Fig. 9. Plot of  $c'_{e,app} \ln \frac{c' - c'_{e,app}}{c'_1 - c'_{e,app}}$  versus  $c_{e,app} \ln \frac{c - c_{e,app}}{c_1 - c_{e,app}}$  for 9 Cabauw net-emission  
1400 experiments. A linear function was fit to each individual dataset and the slope was used to  
1401 calculate the  $\delta D_{soil,app}$  value for each experiment. Errors in x and y direction for each data  
1402 point were considered.

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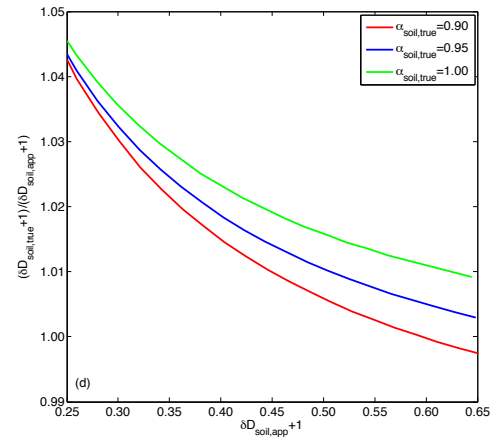
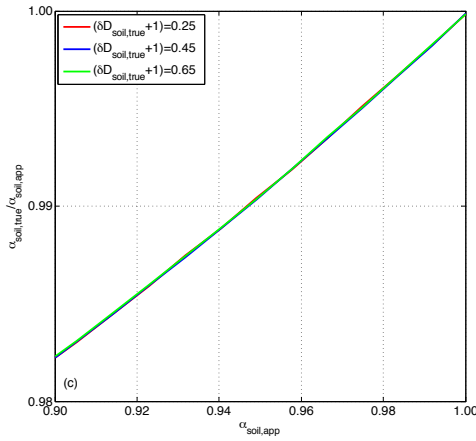
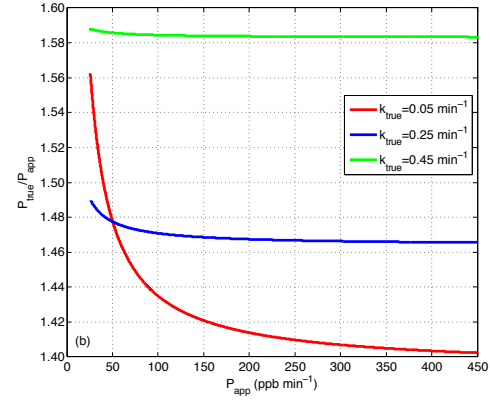
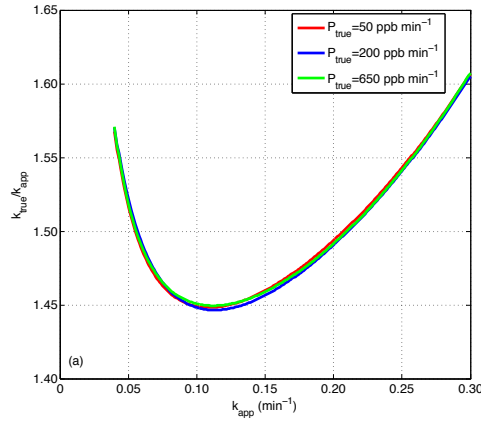


Fig. 10. (a) The relationship between  $k_{\text{true}}/k_{\text{app}}$  and  $k_{\text{app}}$  for  $P_{\text{true}}$  of 50, 200 and 650 ppb min<sup>-1</sup>; (b) between  $P_{\text{true}}/P_{\text{app}}$  and  $P_{\text{app}}$  for  $k_{\text{true}}$  of 0.05, 0.25 and 0.45 min<sup>-1</sup>; (c) between  $\alpha_{\text{soil,true}}/\alpha_{\text{soil,app}}$  and  $\alpha_{\text{soil,app}}$  for  $(\delta D_{\text{soil,true}}+1)$  of 0.25 to 0.65 for  $k_{\text{true}}=0.25$  min<sup>-1</sup> and  $P_{\text{true}}=50$  ppb min<sup>-1</sup>; (d) between  $(\delta D_{\text{soil,true}}+1)/(\delta D_{\text{soil,app}}+1)$  and  $(\delta D_{\text{soil,app}}+1)$  for  $\alpha_{\text{soil,true}}$  of 0.90 to 1.00 for  $k_{\text{true}}=0.25$  min<sup>-1</sup> and  $P_{\text{true}}=50$  ppb min<sup>-1</sup>. The parameters of the sampling setup are  $V'=22.8$  L,  $f=2$  L min<sup>-1</sup>,  $\Delta t=10$  min and the pressures inside the flasks and chamber are 200 kPa and 100 kPa respectively.