

1 Cyanate – a low abundant but actively cycled nitrogen compound in soil

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3 Subtitle: Soil cyanate cycling

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21

22 One-sentence summary

23 Cyanate represents a small but continuously available nitrogen source for soil microbes,
24 contributing to a selective advantage of microorganisms capable of direct cyanate
25 utilization.

26

27 **Abstract**

28 Cyanate (NCO^-) can serve as a nitrogen and/or carbon source for different microorganisms
29 and even additionally as an energy source for autotrophic ammonia oxidizers. Despite the
30 widely distributed genetic potential for direct cyanate utilization among bacteria, archaea
31 and fungi, the availability and environmental significance of cyanate is largely unknown,
32 especially in terrestrial ecosystems. We found relatively low concentrations of soil cyanate,
33 but its turnover was rapid. Contrary to our expectations, cyanate consumption was clearly
34 dominated by biotic processes, and, notably, cyanate was produced *in-situ* at rates similar
35 to that of cyanate formation from urea fertilizer, which is believed to be one of the major
36 sources of cyanate in the environment. Our study provides evidence that cyanate is actively
37 turned over in soils and represents a small but continuous nitrogen/energy source for soil
38 microbes, potentially contributing to a selective advantage of microorganisms capable of
39 direct cyanate utilization.

40

41 **MAIN TEXT**

42

43 **Introduction**

44 Cyanate (NCO^-) is an organic nitrogen compound that has mainly been of interest in medical
45 science due to its negative effect on protein conformation and enzyme activity (e.g., 1), in
46 chemical industry as industrial feedstock, and in industrial wastewater treatment, where it
47 is produced in large amounts, especially during cyanide removal (e.g., 2, 3). However, in
48 recent years, cyanate received more attention in marine biogeochemistry and microbial
49 ecology, with the discovery of the involvement of cyanate in central nitrogen (N) cycling
50 processes, namely in nitrification and anaerobic ammonia oxidation (anammox) (4, 5).

51 Despite the emergent recognition of the role of cyanate in marine ecosystems (6–11), the
52 environmental role and significance of cyanate in terrestrial ecosystems remain entirely
53 unknown.

54 It has been shown that cyanate can serve as the sole N source for microorganisms that
55 encode the enzyme cyanase (also known as cyanate hydrolase or cyanate lyase; EC
56 4.2.1.104) (8, 12, 13). This enzyme catalyzes the decomposition of cyanate in a bicarbonate-
57 dependent reaction yielding carbamate, which spontaneously decarboxylates to ammonia
58 and carbon dioxide (14). The resulting ammonia and carbon dioxide can then be assimilated
59 (13). The enzyme was first discovered in *Escherichia coli* (15) and genes encoding
60 homologous proteins have been found since in genomes of various bacteria, such as
61 proteobacteria and cyanobacteria, as well as in archaea, fungi, plants and animals (16–18).
62 Cyanase, and thus the potential to use cyanate as a N source, therefore seems to be
63 widespread among prokaryotes and eukaryotes. Generally it is assumed that the main role
64 of cyanase is cytoplasmic detoxification. Cyanate is harmful because isocyanic acid
65 (HCNO), the active form of cyanate, reacts with amino and carboxyl groups, and
66 consequently carbamoylates amino acids, proteins and other molecules, thereby altering
67 their structure, charge and function (19). Furthermore, a regulatory function of cyanase in
68 arginine biosynthesis has been proposed (17).

69 Recently, a new physiological role for the enzyme cyanase was described in the
70 chemoautotrophic ammonia-oxidiser *Ca. Nitrososphaera gargensis*. This archaeon encodes
71 a cyanase and was shown to effectively use cyanate not only as a source of N for assimilation
72 but also as a source of energy and reductant (4). Moreover, the marine anammox *Ca.*
73 *Scalindua profunda* as well as several *Ca. Scalindua* single amplified genomes from the
74 Eastern Tropical North Pacific anoxic marine zone also possess a cyanase and it has been
75 suggested that cyanate thus can be directly used as a substrate by anammox organisms (5).

76 Cyanate can be either directly utilized by cyanase-positive microorganisms or indirectly by
77 other microorganisms that may assimilate ammonia released by the former. A special case
78 of indirect use of cyanate was shown recently among nitrifiers exhibiting a reciprocal
79 feeding relationship that enables growth of both partners on cyanate. Cyanase-positive
80 nitrite-oxidizers convert cyanate to ammonia, providing the substrate for cyanase-deficient
81 ammonia oxidizers that oxidize ammonia to nitrite, providing, in turn, the substrate for
82 nitrite-oxidizers (4).

83 Cyanate can be formed by photooxidation or chemical oxidation of hydrogen cyanide (20),
84 or by hydrolysis of thiocyanate (21). Recently, it has also been shown that cyanate is formed
85 in diatom cultures, indicating a biological source of cyanate (22). Within living organisms,
86 cyanate may result from the non-enzymatic decomposition of carbamoyl phosphate, a
87 precursor for nucleotide and arginine biosynthesis (23, 24). Moreover, urea spontaneously
88 dissociates in aqueous solution, forming cyanate and ammonium (25). As urea is the most
89 widely used agricultural N fertilizer worldwide (26), it is possibly one of the most significant
90 sources of cyanate in soils on a global scale.

91 Despite the potential relevance of cyanate as a N and energy source for microorganisms,
92 environmental cyanate sources, concentrations and fluxes (i.e., the production and
93 consumption) are largely unknown, especially in terrestrial ecosystems. Here, we
94 investigated, for the first time, cyanate availability and dynamics in terrestrial ecosystems.
95 We analyzed soil cyanate concentrations across different soil and land management types
96 along a continental gradient and discuss the abiotic behavior of cyanate in the soil
97 environment that controls its availability. We developed a method for compound-specific
98 isotope analysis of cyanate that allowed us to assess biotic and abiotic cyanate turnover
99 processes. To yield further insights into the production and consumption of cyanate in soils,
100 we assessed quantitatively the contribution of urea to soil cyanate formation, by combining

101 empirical and modelling approaches that yielded estimates of gross rates of cyanate
102 transformations in soils.

103

104 **Results and Discussion**

105 *Cyanate concentrations and the influence of soil pH on its recovery and availability*

106 As cyanate concentrations have not yet been determined in soils, we tested three commonly
107 used soil extractants: water (Ultrapure Water, resistivity >18.2 M Ω m), 10 mM CaSO₄, and
108 1 M KCl (Fig. 1). If cyanate is strongly adsorbed in soils, increasing salt concentrations of
109 the extractant result in a higher recovery of cyanate. For an alkaline grassland soil (soil pH
110 = 8.3), we found that the recovery of added cyanate was complete for all extractants (i.e.,
111 no significant difference between added and recovered cyanate, *t*-test, $P > 0.05$). However,
112 the recovery of added cyanate differed between extractants for a forest soil with a soil pH
113 of 7.0 (one-way ANOVA, $F_{2,9} = 308.5$, $P < 0.001$). When using 1 M KCl for this soil,
114 recovery was complete ($101.5\% \pm 1.3$ SE), whereas the use of 10 mM CaSO₄ or water
115 resulted in significantly lower recoveries of 85.8% (± 0.7 SE) and 59.5% (± 1.5 SE),
116 respectively. In contrast to the alkaline and neutral soil, cyanate recovery in an acidic
117 grassland soil was on average only 7% for all extractants. For the following experiments we
118 chose 1 M KCl as the extractant, as its extraction efficiency was the same or higher as the
119 others.

120 To obtain representative data on soil cyanate concentration, we analyzed 46 soils across
121 different soil and land management types along a European climatic gradient (Fig. 2a).
122 Although we used the most sensitive analytical method available to date, with a detection
123 limit in the low nanomolar range in solution (27), cyanate was detectable only in 37% of the
124 soils tested (Fig. 2b). Average concentration of soil cyanate was $33.6 (\pm 8.1$ SE) pmol g⁻¹
125 soil d.w., excluding samples below detection limit. Notably, we found that above soil pH

126 5.7 in 0.01 M CaCl₂ or pH 6.6 in water cyanate was detectable in all samples, indicating
127 that soils with high pH have higher cyanate concentrations, as also shown by the extraction
128 test mentioned above.

129 Soil pH is likely a major factor shaping the availability as well as extractability of cyanate
130 because its reactivity is strongly pH-dependent. Cyanate is the anionic form of isocyanic
131 acid, which is a weak acid with a pK_a of 3.66, so that cyanate is the dominant species at
132 neutral and alkaline pH (Fig. 2c). Based on what has been observed for other inorganic ions,
133 it is predicted that cyanate adsorption in soils decreases with increasing pH, with no
134 adsorption at pH > 8 (Fig. 2c) (28). Such adsorption behavior is in line with the results of
135 our extraction test: at high soil pH, cyanate was completely extracted with water (i.e., no
136 cyanate adsorption), whereas at lower pH (here neutral pH) cyanate extraction was
137 incomplete when extracted with water, but when extracted with salt solutions increasing
138 amounts of cyanate (i.e., exchangeable/adsorbed cyanate) were recovered. In turn, the
139 distinctive low recovery of added cyanate in the acidic soil, as well as the low detectability
140 of cyanate in soils with low pH across a European transect, were most likely due to
141 irreversible reactions of cyanate and in particular isocyanic acid with amino- and carboxyl-
142 groups at low pH. Both chemical species hydrolyze abiotically to ammonia/ammonium and
143 on dioxide/bicarbonate in aqueous solution according to three simultaneous reactions, which
144 are strongly pH-dependent: hydronium ion-catalyzed hydrolysis of isocyanic acid (eq. 6;
145 dominant reaction at low pH), direct hydrolysis of isocyanic acid (eq. 7), and direct
146 hydrolysis of cyanate (eq. 8, dominant reaction at high pH). Combining these reactions, the
147 rate of cyanate/isocyanic acid hydrolysis substantially increases with decreasing pH,
148 rendering cyanate unstable at low pH (markedly at pH < 4; Fig. 2d). Moreover, isocyanic
149 acid also reacts with carboxyl, sulphydryl, phosphate, thiol or phenol groups, which mostly
150 occurs at low pH (29).

151 At neutral to alkaline pH, the most relevant abiotic reactions of cyanate/isocyanic acid in
152 the (soil) environment are the irreversible reaction of isocyanic acid with the amino group
153 of amino acids and proteins (eq. 12; carbamoylation) and the reaction of cyanate and
154 ammonium to urea (eq. 3; equilibrium reaction that favors urea more than 99%). As the rates
155 plotted in Fig. 2d are standardized rates, they do not take into account the concentrations of
156 the two reactants involved in the second order reactions (cyanate and amino acids or cyanate
157 and ammonium). Therefore, the actual rates will depend on the soil solution concentrations
158 of both reactants. Concentrations of amino acids and ammonium in the soil solution are also
159 modulated by their adsorption behavior (i.e., weak or strong), which strongly depends on
160 their chemical properties and on physicochemical properties of the soil, such as clay content
161 and cation exchange capacity (30). Therefore, the rates of abiotic reactions of cyanate with
162 amino acids/proteins or with ammonium may strongly vary between different soil types,
163 depending on soil physicochemical properties other than soil pH. For example, low-nutrient
164 soils with high adsorption capacity for ions and low contents of amino acids and ammonium
165 have the greatest potential to limit these abiotic reactions of cyanate. Nevertheless, cyanate
166 is significantly more stable in soils with high pH, as the rate of abiotic hydrolysis of cyanate
167 to ammonium at pH < 4 is about two orders of magnitude higher compared to the reactions
168 with amino acids or ammonium (note that the standardized rates are plotted on a logarithmic
169 scale in Fig. 2d).

170

171 *Soil cyanate dynamics*

172 Understanding environmental dynamics and turnover of cyanate requires the knowledge
173 about both pool sizes and fluxes. Therefore, we thoroughly assessed cyanate fluxes in
174 neutral/alkaline soils, where it does not rapidly decompose to ammonium, by using two
175 different approaches: first, we determined the half-life ($t_{1/2}$) of cyanate by amending two

176 soils with isotopically labelled cyanate solution ($^{13}\text{C}^{15}\text{N}$ -KOCN) and measuring the
177 decrease in concentration over time. To assess abiotic reactions that may limit cyanate
178 bioavailability in neutral/alkaline soils, we also differentiated between biotic and abiotic
179 decomposition processes of cyanate in this approach using sterilized (autoclaved) soils,
180 where enzymatic activities are strongly reduced. Second, we assessed urea quantitatively as a
181 source for cyanate formation in soils, by combining an empirical and modelling approach
182 to obtain estimates of gross cyanate production and consumption in a urea-amended soil.
183 Throughout the following discussion, we will refer to these two experiments as “tracer
184 experiment” and “urea addition experiment”, respectively.

185 In the tracer experiment, we added isotopically labelled cyanate to two distinct soils with
186 the same pH (7.4 in 0.01M CaCl_2) and similar *in situ* cyanate concentrations: a grassland
187 and an arable soil with soil cyanate concentrations of 27.3 (± 4.7 SE) and 21.2 (± 4.5 SE)
188 pmol g⁻¹ soil d.w., respectively. We found that the depletion of isotopically labelled cyanate
189 was substantially faster in the grassland soil than in the arable soil: 58 (± 2 SD) and 25% (\pm
190 4 SD) of the labelled cyanate were lost in the grassland and arable soil, respectively, after
191 90 min of incubation. Here, the depletion of cyanate includes both biotic and abiotic
192 processes. To distinguish abiotic reactions and biotic cyanate consumption over time, we
193 corrected these data for abiotic cyanate loss rates inferred from sterile (autoclaved) soil
194 samples. We then fitted a first order exponential decay curve and used the exponential
195 coefficient to calculate the biotic half-life of cyanate. We found that the grassland soil had
196 a biotic half-life of 1.6 h, which is significantly shorter than that of the arable soil, which
197 was 5.0 h ($t = 6.64$, $P < 0.01$; Fig. 3). This biotic-mediated turnover of the soil cyanate pool
198 was relatively fast and in the same range as the turnover of free amino acids in soils and
199 plant litter (< 6h) (31, 32) and soil glucosamine (33). By contrast, mean residence times of
200 soil ammonium and nitrate are found to be around 1 day (half-life of 16.6 h), but can also

201 be in the range of several days due to lower input rates and larger pool sizes. For instance,
202 in arable soils ammonium and nitrate had mean residence times between 0.6 and 7.9 day
203 (half-life of 10.0 h to 5.5 d), and between 1.1 and 25.7 d (half-life of 18.3 h to 17.8 d),
204 respectively (34). The abiotic half-life of cyanate determined in sterile soil samples was
205 similar for both soils ($t = 0.13$, $P = 0.9024$), with 13.4 h and 15.1 h for the grassland and the
206 arable soil, respectively (Fig. 3). The ratio of the biotic (k_b ; min^{-1}) and abiotic (k_a ; min^{-1}) rate
207 constant of cyanate consumption was 8 ($k_b/k_a = 0.007/0.0009$) for the grassland soil and 3
208 ($k_b/k_a = 0.002/0.0008$) for the arable soil. This shows that the consumption of cyanate in
209 these neutral/alkaline soils is mainly biotic, with only small contributions from abiotic
210 processes.

211 The contribution of urea to soil cyanate formation has never been quantified, although it has
212 been speculated that cyanate formation is the reason for the observed negative effects of
213 urea fertilizer (when applied at high rates) on early plant growth (35). It was found that
214 cyanate was toxic to plant cells, although when cyanate was added to soil, it did not have a
215 negative effect on seed germination and plant biomass yield (35, 36). Nevertheless, it is
216 unclear whether cyanate accumulates during fertilizer application, and urea-derived cyanate
217 has never been considered in the context of microbial nutrient cycling in agricultural soils.
218 Studying cyanate formation from urea fertilizer application in soils has been hindered by
219 the lack of sensitive analytical methods to measure cyanate in the environment, which has
220 only recently become available (27). This is also complicated by the fact that rates of cyanate
221 formation from urea in soils depend on the pool sizes of different N species, which, in
222 contrast to sterile aqueous solutions under laboratory conditions, change over time. These
223 changes are due to microbial activity, i.e., decrease in urea concentration due to ureolytic
224 activity, net change in ammonium concentration as a result of the production from urea
225 hydrolysis and organic matter mineralization, and the consumption and/or immobilization

226 by nitrification, assimilation and soil fixation (abiotic immobilization by clay and humic
227 substances), and the biotic consumption of cyanate.

228 In order to obtain estimates of gross rates of cyanate dynamics, we developed an approach
229 that combines experimental data and modelling. The chemical equilibrium reaction of urea
230 and ammonium cyanate has been intensively studied and the rate constants for this reaction
231 in aqueous solution are well established under controlled laboratory conditions (eq. 3-5).
232 We took advantage of these well-established rate constants by using them to compute rates
233 of cyanate production and consumption based on observed changes in pool sizes in soil
234 solution (eq. 14 and Fig. 4a). We assume that net changes in cyanate concentration are the
235 result of the production from urea and the biotic and abiotic consumption of cyanate, and
236 that no cyanate adsorption occurs in the alkaline soil used in this experiment.

237 For this “urea addition experiment” we used the same arable soil as in the tracer experiment,
238 which was cultivated with rice every second year and received N fertilizer in the form of
239 urea. Urea solution corresponding to the fertilizer application rate of this soil (i.e., 180 kg N
240 $\text{ha}^{-1} \text{y}^{-1}$) was added, and soil solutions were obtained at several time points throughout a 30-
241 h incubation period. We found that urea was almost completely hydrolyzed at the end of the
242 incubation (Fig. 4b), and that only a very small fraction (<1%) of the resulting ammonium
243 was recovered in soil solution throughout the incubation (Fig. 4c). Thus, most of the
244 ammonium was adsorbed, abiotically fixed, converted to nitrate or assimilated. When urea
245 was added to the soil incubations at the beginning, a small cyanate amount was added along
246 with it. This was unavoidable as cyanate was immediately formed upon urea dissolution
247 when the solution was prepared. This cyanate pool was rapidly consumed during the first 6
248 h, after which steady cyanate concentrations were reached, indicating balanced production
249 and consumption rates (Fig. 4d). The rate of cyanate formation from urea depends on the
250 pool size of urea, ammonium and cyanate, which change over time (i.e., decrease of urea

concentration due to ureolytic activity, while net changes in ammonium concentration are the result of the production from urea hydrolysis and the consumption and ammonium immobilization by nitrification and fixation/assimilation, respectively). For the model, urea concentration over time was described by a first order reaction (eq. 15), and ammonium and cyanate concentrations were fitted with a third and fourth degree polynomial function, respectively (eq. 16 and 17, respectively). By integrating dynamics of biological processes into the abiotic equilibrium reactions of urea (eq. 14), our model estimates cyanate production of 86.8 nM from urea (180 kg N ha^{-1}) after 30 h (Fig. 4e), which equals to an average gross cyanate production rate from urea of 2.9 nM h^{-1} . Gross cyanate consumption was 6.0 nM h^{-1} (180 nM during 30 h), encompassing also the consumption of the added cyanate through urea addition at the beginning of the incubation. Our study therefore demonstrates that cyanate formed by isomerization of urea was rapidly depleted by soil microorganisms and by abiotic reactions, limiting cyanate accumulation in soils and, thus preventing possible phytotoxic effects of urea-derived cyanate during fertilizer application. The applied empirical modelling approach provides the first estimates of gross cyanate production and consumption rates from urea in a biological/environmental system.

To better grasp the cyanate consumption potential of soil microorganisms, we compared the rate constant of cyanate consumption from the tracer experiment and urea hydrolysis from the urea addition experiment, as both rates followed first order reaction kinetics (Fig. 3b and Fig. 4b, respectively). In the arable soil used for both experiments, we obtained a rate constant of 0.0032 min^{-1} for (biotic) cyanate degradation and 0.0009 min^{-1} for urea hydrolysis, showing that cyanate consumption was approximately 3.7-fold faster than urea hydrolysis. This indicates that soil microorganisms have a remarkably high potential for cyanate consumption, especially by comparison with the well-known rapid hydrolysis of urea in soils due to high ureolytic activity.

276 However, knowing how much cyanate is continuously produced *in-situ* in (not urea
277 amended) soils is still unsolved. Soil cyanate concentrations were too low for performing
278 an isotope pool dilution assay to determine gross rates of cyanate production and
279 consumption. We therefore explored *in-situ* gross cyanate production rates by an alternative
280 approach. We used concentrations and mean residence times (MRT) of cyanate in soils to
281 calculate gross cyanate production rates assuming steady-state conditions, i.e., productive
282 and consumptive fluxes are balanced, giving a zero net change in cyanate concentration, for
283 an unamended soil ($flux = pool/MRT$). For the urea addition experiment, we computed
284 MRTs of cyanate for 6 h-time intervals, which ranged between 3.9 to 20.9 h, with lower
285 MRTs at the beginning of the incubation (Table 1). For the tracer experiment, where we
286 added isotopically labelled cyanate, we calculated half-life of cyanate that includes both
287 abiotic and biotic processes for the arable soil ($t_{1/2} = 3.6h$) and converted it to MRT (MRT
288 $= t_{1/2}/0.693$), which was 5.2 h (Table 1). This MRT is in the same range as the MRTs
289 computed for the first 12 h of the urea addition experiment. Using the MRT of 5.2 h derived
290 from the tracer addition experiment and the *in-situ* cyanate concentration of this soil (21.2
291 pmol g⁻¹ d.w.), we obtained a gross cyanate production rate of 98.8 pmol g⁻¹ d.w. d⁻¹. This
292 gross cyanate production rate was approximately 4-times higher than the rate at which
293 cyanate is formed through isomerization of urea (26.0 pmol g⁻¹ d.w. d⁻¹; Table 1). However,
294 additions of substrates can stimulate consumptive processes and, thus, can lead to an
295 overestimation of fluxes in relation to unamended conditions, which consequently results in
296 lower MRTs. Assuming that the MRT derived from the tracer experiment as well as MRTs
297 computed for the first 12 h of the incubation with urea are underestimated due to the
298 substrate addition, we further calculated conservative estimates of gross cyanate production
299 rates, using MRTs of 24 h (which is similar to the MRT for the end of the incubation with
300 urea, when the initial pulse of cyanate was depleted), and 48 h. This yielded gross cyanate

301 production rates of 21.2 and 10.6 pmol g⁻¹ d.w. d⁻¹, respectively. These rate estimates are
302 still in the same order of magnitude as the average cyanate gross production rate during the
303 30-h incubation with urea (26.0 pmol g⁻¹ d.w. d⁻¹; Table 1). These rates are more than 3
304 orders of magnitude lower than gross rates of N mineralization and nitrification in soils (37)
305 and approximately 1-2 orders of magnitude lower than gross production rates of some
306 organic N compounds from microbial cell wall decomposition in soils (33). While our
307 calculations do not necessarily represent accurate estimates of *in-situ* gross cyanate
308 production rates, they provide a first approximation of their magnitude in soils, as
309 environmental cyanate production rates are entirely unknown. Most importantly, our data
310 thus suggest that cyanate in unamended soils may be produced at rates similar to rates of
311 cyanate formation from urea fertilizer.

312 Sources of cyanate in natural ecosystems are not well understood. It is possible that, in
313 natural/uncontaminated soils, cyanate is formed from cyanide, which can be released by
314 cyanogenic bacteria, fungi and plants into the soil (38, 39). Another source of cyanate can
315 be urea excreted by soil fauna or released by lysed microbes. Soil urea concentrations are in
316 the low nmol g⁻¹ range (40), being about 3 orders of magnitude higher than soil cyanate
317 concentrations. Furthermore, within living organisms, cyanate may result from the non-
318 enzymatic decomposition of carbamoyl phosphate, a nucleotide precursor (23), which may
319 leak into the environment during growth or lysis of an organism. It has been shown that net
320 cyanate production occurred in diatom cultures during the stationary phase, but not in a
321 cyanobacterial culture (22). However, the pathway of cyanate production in these diatom
322 cultures is unknown. This certainly warrants future work, especially because cyanate
323 production through the repetitive process of organisms' growth and death would provide a
324 continuous source of cyanate in the environment.

326 *Cyanate availability across different environments*

327 The cyanate concentrations measured in the soils studied here were low compared to other
328 N pools. The abundance of cyanate was about 3 orders of magnitude lower than ammonium
329 or nitrate in the soils across a European transect. To determine if cyanate concentrations are
330 exceptionally low in soils in general, we compared cyanate concentrations across different
331 environments. As cyanate concentrations are largely unknown in other environments, we
332 analyzed cyanate in salt marsh sediments including pore water, and activated sludge as well
333 as discharge from municipal wastewater treatment plants. We additionally collected
334 published data on marine cyanate concentrations (22). As direct comparisons of cyanate
335 concentrations are not possible due to different matrices (seawater, soil extracts, pore water),
336 we normalized cyanate concentrations by calculating ammonium-to-cyanate ratios.
337 Ammonium is a major N source in the environment and can be used as an indicator of the
338 N status of an ecosystem, and, thus, this ratio can be interpreted as a proxy of relative
339 cyanate-N availability. The median of ammonium-to-cyanate ratios was 955 for soil
340 extracts, 1842 for salt marsh sediment extracts, 606 for pore water extracted from salt marsh
341 sediments, 2189 and 514 for activated sludge and discharge of wastewater treatment plants,
342 respectively, and 14 for seawater (Fig. 5). Despite large differences between median values
343 between some environments, we found no significant differences in relative cyanate
344 availability between soils and any of the other environments, except for seawater, which
345 had lower ammonium-to-cyanate ratios (Kruskal-Wallis test followed by Dunn's test, $H(2)$
346 $= 101.1$, $P < 0.001$). These results indicate that relative cyanate concentrations in soils are
347 similar to those in salt marsh sediments or activated sludge from wastewater treatment
348 plants. Seawater showed the lowest ammonium-to-cyanate ratios, which were significantly
349 lower than for all other environments. Cyanate concentrations in seawater are in the
350 nanomolar range, which is in the same order of magnitude as ammonium concentrations

351 typically found in oligotrophic marine environments (22, 27, 41). In contrast to the low
352 MRT of cyanate in soils, that of cyanate in marine surface water has been shown to range
353 between 2.3 d and 8.1 d (similar to MRT of ammonium) but can be as high as 36 d (41).
354 Therefore, in marine systems relative concentrations of cyanate are higher but cyanate
355 turnover rates are slower than in terrestrial systems.

356

357 Conclusion

358 Soil is a heterogenous environment in regard to its physicochemical properties, and thus
359 assessing cyanate bioavailability requires a thorough analysis of the abiotic and biotic
360 behavior of cyanate. Although neutral/alkaline soil pH favors cyanate stability, it may also
361 be interesting to specifically look at low pH soils with detectable cyanate concentrations, as
362 the faster abiotic decomposition needs to be compensated by higher production rates.
363 Although soil cyanate concentrations may seem quantitatively insignificant compared to
364 those of ammonium, cyanate may constitute an important, yet largely overlooked, N and
365 energy source for soil microorganisms, specifically when considering the relatively high
366 production rates. Additionally, cyanate is more mobile in soil solution compared to
367 ammonium, the availability of which is strongly limited in soils through adsorption,
368 favoring the relative availability of cyanate-N in soil solution. Using cyanate directly as a
369 source of energy, carbon dioxide or nitrogen could thus represent a selective advantage for
370 specific microbial taxa. The ability to use cyanate as a source of reductant (i.e., ammonia)
371 and carbon (i.e., carbon dioxide) may also be an important ecological adaptation of
372 ammonia-oxidizing microorganisms, with implications for soil nitrification. Although only
373 a few genomes of ammonia-oxidizing archaea and complete ammonia-oxidizing
374 (comammox) organisms are known to encode cyanases (5, 42, 43), another but yet unknown
375 enzyme may be involved in the decomposition of cyanate. Kitzinger et al. (7) found that an

376 isolate of a marine ammonia-oxidizing archaeon lacking a cyanase can oxidize cyanate to
377 nitrite. Furthermore, consortia of cyanase-encoding nitrite-oxidizers and non-cyanase
378 encoding ammonia oxidizers can collectively thrive on cyanate as energy source (4).
379 Clearly, the fate of cyanate-N in soils needs to be further investigated, together with the
380 microbial populations that are involved in cyanate turnover or are able to use cyanate
381 directly as a N and energy source. Our study provides a first insight into cyanate dynamics
382 in soils, providing evidence that cyanate is actively turned over in soils and represents a
383 small but continuous N source for soil microbes.

384

385 **Materials and Methods**

386 *Cyanate analysis*

387 To test soil extractants for cyanate analysis, three soils (0-15 cm depth) differing in soil pH
388 were collected in Austria, sieved to 2 mm and stored at 4°C. An alkaline grassland soil was
389 collected in the National Park Seewinkel (47° 46' 32" N, 16° 46' 20" E; 116 m a.s.l.), a
390 neutral mixed forest soil in Lower Austria (N 48° 20' 29" N, 16° 12' 48" E; 171 m a.s.l.)
391 and an acidic grassland soil at the Agricultural Research and Education Centre Raumberg-
392 Gumpenstein (47° 29' 45" N, 14° 5' 53" E; 700 m a.s.l.). The recovery of cyanate was
393 assessed by using cyanate-spiked (15 nM potassium cyanate added) and unspiked extraction
394 solutions. We used water (Milli-Q, >18.2 MΩ, Millipore), 10 mM CaSO₄ and 1 M KCl
395 as extractants. The three soils (n=4) were extracted using a soil:extractant ratio of 1:10 (w:v),
396 shaken for 10 min, and centrifuged (5 min at 14000 × g). The supernatant was stored at -
397 80°C until analysis, as it has been shown that cyanate is more stable at -80°C compared to
398 -20°C (27).

399 To explore soil cyanate concentrations across different soil and land management types, and
400 along a climatic gradient, we collected 42 soils from Europe. Sites ranged from Southern

401 France to Northern Scandinavia and included forests (F), pastures (P), and arable fields (A)
402 (Fig. 2a). At each site five soil cores (5 cm diameter, 15 cm depth) were collected, after
403 removal of litter and organic horizons. Soil samples were shipped to Vienna and aliquots of
404 the five mineral soil samples of each site were mixed to one composite sample per site and
405 sieved to 2 mm. In addition to those 42 samples, we collected a rice paddy soil in Southern
406 France (sample code A1; four replicates) and three grassland soils (G) in close vicinity of
407 Vienna, Austria (G1 and G2 from saline grassland, three replicates; G3, one soil sample).
408 Soil samples were stored at 4°C and extracted within a few days. All sampling sites with
409 their location, soil pH, and cyanate, ammonium and nitrate concentrations are listed in Table
410 S1. For cyanate and ammonium analysis, soils (2 g fresh soil) were extracted with 15 mL 1
411 M KCl, shaken for 30 min and centrifuged (2 min at 10000 × g). The supernatants were
412 transferred to disposable 30 mL syringes and filtered through an attached filter holder
413 (Swinnex, Millipore) containing a disc of glass microfiber filter (GF/C, Whatman). To
414 reduce abiotic decay of cyanate to ammonium during extraction, the extraction was
415 performed at 4°C with the extracting solution (1 M KCl) cooled to 4°C prior to extraction.
416 Soil extracts were stored at -80°C until analysis.

417 To compare cyanate availability across different environments, we analyzed cyanate in salt
418 marsh sediments and activated sludge from municipal wastewater treatment plants, and,
419 additionally, we collected published data on cyanate concentrations in the ocean. We
420 collected sediment samples (0-10 cm, n=4) from a high and low salt marsh dominated by
421 *Spartina alterniflora* in New Hampshire, USA (43° 2' 26" N, 70° 55' 36" W), and from a
422 *S. alterniflora* and a *S. patens* salt marsh in Maine, USA (43° 6' 31" N, 70° 39' 56" W). We
423 chose these types of salt marsh because they have been shown to accumulate cyanide (44),
424 which potentially could be oxidized to cyanate. Sediment samples were stored at 4°C and
425 extracted within a few days after collection using 2 M KCl at a sediment:extractant ratio of

426 1:10 (w:v) for 30 min at room temperature. The supernatants were filtered through glass
427 microfibre filters as described above for soil samples. Pore water was extracted with Rhizon
428 samplers (Rhizon CSS, 3 cm long, 2.5 mm diameter, Rhizosphere Research Products,
429 Netherlands) with a filter pore size of 0.15 μm . Triplicate samples of activated sludge were
430 collected from four municipal Austrian wastewater treatment plants (WWTPs), i.e., from
431 Alland ($48^\circ 2' 30''$ N, $16^\circ 6' 1''$ E), Bruck an der Leitha ($48^\circ 2' 4''$ N, $16^\circ 49' 7''$ E),
432 Wolkersdorf ($48^\circ 21' 31''$ N, $16^\circ 33' 31''$ E) and Klosterneuburg ($48^\circ 17' 39''$ N, $16^\circ 20' 30''$ E). Samples from the discharge were also collected from the first three listed WWTPs.
433 Samples were cooled on gel ice packs during the transport to Vienna. Upon arrival in
434 Vienna, samples were transferred to disposable 30 mL syringes and filtered through an
435 attached filter holder (Swinnex, Millipore) containing a disc of glass microfiber filter (GF/C,
436 Whatman). All samples were immediately stored at -80°C until analysis.
437

438 Cyanate concentrations were determined using high performance liquid
439 chromatography (HPLC) with fluorescence detection, after conversion to 2,4(1H,3H)-
440 quinazolinedione (27). Briefly, a 230 μL aliquot of the sample was transferred to a 1.5 mL
441 amber glass vial, 95 μL of 30 mM 2-aminobenzoic acid (prepared in 50 mM sodium acetate
442 buffer, pH = 4.8) were added, and samples were incubated at 37°C for 30 min. The reaction
443 was stopped by the addition of 325 μL of 12 M HCl. Standards (KOCN) were prepared
444 fresh daily and derivatized with samples in the same matrix. Derivatized samples were
445 frozen at -20°C until analysis. Just before analysis samples were neutralized with 10 M
446 NaOH. The average detection limit was 1.2 nM (± 0.2 SE). Ammonium concentrations were
447 quantified by the Berthelot colorimetric reaction. As direct comparison of cyanate
448 concentrations was not possible across the different environments and matrices, we
449 normalized cyanate concentrations relative to ammonium concentrations, by calculating
450 ammonium-to-cyanate ratios. Data on marine cyanate and ammonium concentrations were

451 taken from Widner et al. (22). For marine samples where cyanate was detectable but
452 ammonium was below detection limit, we used the reported limit of detection of 40 nM for
453 ammonium. The presented soil and sediment data are biased towards higher cyanate
454 availabilities (i.e., low $\text{NH}_4^+/\text{NCO}^-$ ratios), due to the exclusion of samples where cyanate
455 was possibly present but was below detection limit. Soil pH was measured in 1:5 (w:v)
456 suspensions of fresh soil in 0.01 M CaCl_2 and water.

457

458 *Dynamics of cyanate consumption in soil using stable isotope tracer*

459 For the determination of half-life of cyanate, we used two soils: a grassland soil (G3) and a
460 rice paddy soil (A1). Both soils had a pH of 7.4 (determined in 0.01 M CaCl_2). The grassland
461 soil had a soil organic C content of 3.7%, soil N content of 0.192%, molar C:N ratio of 22.4,
462 ammonium concentration of 5.60 nmol g⁻¹ d.w., nitrate concentration of 1.03 $\mu\text{mol g}^{-1}$ d.w.,
463 and an electrical conductivity of 82.0 mS/m. The rice paddy soil had a soil organic C content
464 of 1.0%, soil N content of 0.098%, molar C:N ratio of 11.9, ammonium concentration of
465 2.47 nmol g⁻¹ d.w., nitrate concentration of 0.91 $\mu\text{mol g}^{-1}$ d.w., and an electrical conductivity
466 of 21.7 mS/m. To equilibrate soil samples after storage at 4°C, soil water content was
467 adjusted to 55% water holding capacity (WHC) and soils incubated at 20°C for one week
468 prior to the start of the experiment. To correct for abiotic reactions of cyanate, a duplicate
469 set of soil samples was prepared and one set of them was sterilized by autoclaving prior to
470 label addition while the other set was left under ambient conditions. Soil samples were
471 autoclaved three times at 121°C for 30 min with 48 h-incubations at 20°C between
472 autoclaving cycles to allow spores to germinate prior to the next autoclaving cycle and to
473 inactivate enzymes (45).

474 Preliminary experiments indicated rapid consumption of added cyanate. Thus, to avoid fast
475 depletion of the added cyanate pool, we added 5 nmol $^{13}\text{C}^{15}\text{N-KOCN}$ g⁻¹ f.w. (^{13}C : 99

476 atom%; ^{15}N : 98 atom%), which equals to approximately 250-fold the *in-situ* cyanate
477 concentration. With the tracer addition the soil water content was adjusted to 70% WHC.
478 After tracer addition, non-sterile and sterile soil samples were incubated at 20°C for a period
479 of 0, 10, 20, 30, 45, 60 and 90 min (n=3) before stopping the incubation by extraction. Soil
480 extractions were performed with 1 M KCl as described above for the 46 soil samples. Soil
481 extracts were stored at -80°C until analysis.

482 As no method for compound-specific isotope analysis of cyanate existed, we developed a
483 method to measure isotopically labelled and unlabeled forms of cyanate in soil extracts
484 using hydrophilic interaction chromatography coupled to high-resolution electrospray
485 ionization mass spectrometry (HILIC-LC-MS). For this analysis, cyanate was converted to
486 2,4(1*H*,3*H*)-quinazolinedione as described above for the RP-HPLC method but with some
487 modifications. Aliquots of 280 μL of each sample were transferred to 2 mL plastic reaction
488 vials, and 20 μL of internal standard solution (4 μM ^{13}C -KOCN, 98 atom%) were added.
489 To start the reaction, 120 μL of 30 mM 2-aminobenzoic acid (prepared in ultrapure water)
490 were added, and samples were incubated at 37°C for 30 min. The reaction was stopped by
491 the addition of 420 μL 12 M HCl. To remove HCl and bring the target compound into an
492 organic solvent that can be easily evaporated, we performed liquid-liquid extractions using
493 a mixture of ethyl acetate/toluene (85/15 (v/v)). Each sample was extracted 3 times with 1
494 mL organic solvent mixture. For extraction, samples were thoroughly mixed by vortexing
495 and the tubes were briefly spun down to separate the two phases. The organic phases of each
496 extraction were combined in a 10 mL amber glass vial and dried under a stream of N_2 .
497 Before analysis, samples were redissolved in 200 μL mobile phase. Samples were analyzed
498 on a UPLC Ultimate 3000 system (Thermo Fisher Scientific, Bremen, Germany) coupled to
499 an Orbitrap Exactive MS (Thermo Fisher Scientific). 2,4(1*H*,3*H*)-quinazolinedione was
500 separated using an Accucore HILIC column (150 mm \times 2.1 mm, 2.6 μm particle size) with

501 a preparative guard column (10 mm × 2.1 mm, 3 μ m particle size; Thermo Fisher Scientific).
502 We used isocratic elution with 90/5/5 (v/v/v) acetonitrile/methanol/ammonium acetate, with
503 a final concentration of ammonium acetate of 2 mM (pH = 8). The sample injection volume
504 was 7 μ L, and the flow rate 0.2 mL min⁻¹. The Orbitrap system was used in negative ion
505 mode and in full scan mode at a resolution of 50,000. The source conditions were: spray
506 voltage 4 kV, capillary temperature 275°C, sheath gas 45 units, and AUX gas 18 units. The
507 instrument was calibrated in negative ion mode before sample acquisition using Pierce LTQ
508 ESI Negative Ion Calibration Solution (Thermo Fisher Scientific). To improve the accuracy
509 of absolute quantification, external calibration was paired with an internal calibrant (¹³C-
510 potassium cyanate) to correct for deviations in liquid-liquid extraction efficiency, ionization
511 efficiency and ion suppression. ¹³C-KOCN (98 atom%) and ¹³C¹⁵N-KOCN (¹³C: 99 atom%;
512 ¹⁵N: 98 atom%) were purchased from ICON Isotopes. The mass-to-charge (*m/z*) ratio of
513 unlabeled, ¹³C- and ¹³C¹⁵N-labelled cyanate was 161.0357, 162.0391, and 163.0361,
514 respectively, and the retention time was 2.2 min. The limit of detection was 9.7 nM.

515 To obtain biotic cyanate consumption rates, the non-sterile samples were corrected for
516 abiotic decomposition of cyanate derived from the sterile (autoclaved) samples. Dynamics
517 of cyanate consumption over time for the corrected non-sterile soils were then described by
518 fitting a first order exponential decay curve:

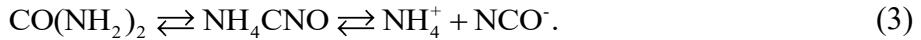
519
$$C(t) = C_0 e^{(-kt)}, \quad (1)$$

520 Where $C(t)$ is the remaining ¹³C¹⁵N-cyanate concentration at time t , C_0 is the initial
521 concentration of ¹³C¹⁵N-cyanate and k is the exponential coefficient for ¹³C¹⁵N-cyanate
522 consumption. The half-life ($t_{1/2}$) of the ¹³C¹⁵N-cyanate pool was calculated as:

523
$$t_{1/2} = \frac{\ln(2)}{k}. \quad (2)$$

525 *Abiotic reactions of cyanate and isocyanic acid*

526 Urea ($\text{CO}(\text{NH}_2)_2$) exists in chemical equilibrium with ammonium cyanate (NH_4CNO) in
527 aqueous solution:



529 The rate constant for the decomposition of urea (k_{1a}) and for the conversion of ammonium
530 cyanate into urea (k_{1b}) were taken from Hagel et al. (46), and temperature dependence was
531 calculated by using the Arrhenius equation:

532
$$k_{1a} = 1.02 \times 10^{16} e^{-16006/T} \text{ (min}^{-1}\text{)},$$
 (4)

533
$$k_{1b} = 4.56 \times 10^{13} e^{-11330/T} \text{ (M}^{-1} \text{ min}^{-1}\text{)},$$
 (5)

534 where T is temperature in Kelvin.

535 Cyanate is the anionic form of isocyanic acid. The latter exists as two isomers in aqueous
536 solution, where isocyanic acid is the dominant species. Thus, the acid will be referred to as
537 isocyanic acid. The decomposition of isocyanic acid and cyanate in aqueous solution was
538 found to take place according to three simultaneous reactions:



542 Eq. (6) is for the hydronium ion catalyzed hydrolysis of isocyanic acid (rate constant k_{2a} ;
543 dominant reaction at low pH), eq. (7) is for the direct hydrolysis of isocyanic acid (k_{2b}), and
544 eq. (8) is for the direct hydrolysis of cyanate (k_{2c} ; dominant reaction at high pH). The rate
545 constants are as follows (46):

546
$$k_{2a} = 3.75 \times 10^{11} e^{-7382/T} \text{ (M}^{-1} \text{ min}^{-1}\text{)},$$
 (9)

547 $k_{2b} = 1.54 \times 10^{10} e^{-7637/T} \text{ (min}^{-1}\text{)},$ (10)

548 $k_{2c} = 2.56 \times 10^{11} e^{-11933/T} \text{ (min}^{-1}\text{).}$ (11)

549 Isocyanic acid reacts with amino groups of proteins, in a process called carbamoylation (19):



551 We used glycine as an example for an amino acid, with the following rate constant (47):

552 $k_3 = 8.68 \times 10^{15} e^{-8008/T} \text{ (M}^{-1}\text{min}^{-1}\text{).}$ (13)

553

554 *Urea-derived cyanate formation in a fertilized agricultural soil*

555 For studying the formation and consumption of cyanate after urea addition, we used a rice
556 paddy soil (A1; the same soil as used in the stable isotope tracer experiment), which was
557 cultivated with rice once every second year with a urea application rate of 180 kg N ha⁻¹ y⁻¹. Treatment of the soil samples was the same as for the stable isotope tracer experiment.
558 Briefly, soil water content was adjusted to 55% water holding capacity (WHC) and soil
559 samples (4 g of fresh soil in a 5 mL centrifugation tube) were incubated at 20°C for one
560 week prior to the start of the experiment. With the addition of the urea solution, the soil
561 water content was adjusted to 70% WHC. We added 140 µg urea g⁻¹ soil d.w., which
562 corresponds to approximately 180 kg N ha⁻¹. Soil samples were incubated at 20°C for a
563 period of 0, 6, 12, 24 and 30 h (n=4). At each sampling, we collected the soil solution. For
564 this a hole was pierced in the bottom of the 5 mL centrifugation tube containing the soil
565 sample. This tube was then placed into another, intact, 15 mL centrifugation tube and this
566 assembly was then centrifuged at 12000 × g for 20 min at 4°C to collect the soil solution.
567 Soil solution samples were stored at -80°C until analysis. For comparative analysis, we
568 converted rates based on nmol/L soil solution to rates based on a dry soil mass basis. For

570 the conversion, we recorded the volume of the soil solution collected and determined the
571 water content of the soil samples after centrifugation.

572 Cyanate concentrations in soil solution were determined as described above using HPLC.
573 Urea was quantified by the diacetyl monoxime colorimetric method, ammonium by the
574 Berthelot colorimetric reaction and ammonium, and nitrite and nitrate by the Griess
575 colorimetric procedure. For cyanate analysis, aliquots of two replicates were pooled because
576 of insufficient sample volume.

577 We used the well-established rate constants for the equilibrium reaction of urea in aqueous
578 solution and decomposition of cyanate to ammonia/ammonium and carbon
579 dioxide/bicarbonate, to model gross cyanate production and consumption after urea
580 amendment from observed changes in urea, ammonium and cyanate concentrations over
581 time. Cyanate accumulation was calculated as cyanate formation from urea (rate constant
582 k_{1a} , eq. 4) minus the conversion of ammonium cyanate into urea (rate constant k_{1b} , eq. 5),
583 and minus abiotic cyanate hydrolysis to ammonium and carbon dioxide (rate constants k_{2a} ,
584 k_{2b} , k_{2c} , eq. 9-11). It has been found that only the ionic species (i.e., NCO^- and NH_4^+) are
585 involved in the reaction of ammonium cyanate to urea. The difference between cyanate
586 accumulation and the net change in cyanate concentration over time gives then cyanate
587 consumption, as follows:

$$\frac{d[\text{consumed } \text{NCO}^-]}{dt} = k_{1a}[\text{CO}(\text{NH}_2)_2] - k_{1b} \left(\frac{K_{\text{HNCO}}[\text{NCO}^-]}{K_{\text{HNCO}} + [\text{H}_3\text{O}^+]} \right) \left(\frac{[\text{H}_3\text{O}^+][\text{NH}_4^+]}{K_{\text{NH}_3} + [\text{H}_3\text{O}^+]} \right) - \left(k_{2a}[\text{H}_3\text{O}^+] \left(\frac{[\text{H}_3\text{O}^+][\text{NCO}^-]}{K_{\text{HNCO}} + [\text{H}_3\text{O}^+]} \right) + k_{2b} \left(\frac{[\text{H}_3\text{O}^+][\text{NCO}^-]}{K_{\text{HNCO}} + [\text{H}_3\text{O}^+]} \right) + k_{2c} \left(\frac{K_{\text{HNCO}}[\text{NCO}^-]}{K_{\text{HNCO}} + [\text{H}_3\text{O}^+]} \right) \right) - [\text{NCO}^-], \quad (14)$$

589 where $[\text{NCO}^-]$ represents the concentration of cyanate and isocyanic acid, $[\text{NH}_4^+]$ is the sum
590 of ammonium and ammonia, K_{HNCO} and K_{NH_3} is the acid dissociation constant of isocyanic
591 acid and ammonia, respectively, and $[\text{H}_3\text{O}^+]$ is the hydronium ion concentration. Urea

592 concentration over time was described by a first order reaction (eq. 15; unit of rate constant
593 is min^{-1}), and ammonium and cyanate concentrations were fitted with a third and fourth
594 degree polynomial function, respectively (eq. 16 and 17, respectively), as follows:

595
$$\frac{d[\text{CO}(\text{NH}_2)_2]}{dt} = 8.64 \times 10^{-4} [\text{CO}(\text{NH}_2)_2], \quad (14)$$

596
$$\frac{d[\text{NH}_4^+]}{dt} = 2.74 \times 10^{-13} t^2 - 3.52 \times 10^{-10} t + 8.04 \times 10^{-8}, \quad (15)$$

597
$$\frac{d[\text{NCO}^-]}{dt} = 3.47 \times 10^{-19} t^3 - 1.20 \times 10^{-15} t^2 + 1.31 \times 10^{-12} t - 4.41 \times 10^{-10}, \quad (16)$$

598 where t is time in min and concentrations are mol/L soil solution.

599 The input parameters were 7.4 for pH (pH of solution: 7.4 ± 0.1 SD) and 20°C for
600 temperature. As rate constant k_{1b} is dependent on the ionic strength, we corrected the rate
601 constant (given at $I = 0.25$ (46)) using the Extended Debye-Hückel expression:

602
$$-\log f = \frac{Az^2 \sqrt{I}}{I + aB\sqrt{I}}, \quad (17)$$

603 Where f is the activity coefficient, A and B are constants that vary with temperature (at 20°C,
604 $A = 0.5044$ and $B = 3.28 \times 10^8$), z is the integer charge of the ion, and a is the effective
605 diameter of the ion ($a = 5 \text{ \AA}$; , 46). We used an ionic strength $I = 0.01$, which is within the
606 range observed for soils.

607

608 *Statistical Analysis*

609 Statistical significance of the difference between extractants within each soil type was
610 analyzed by one-way analysis of variance (ANOVA) followed by Tukey HSD post-hoc test.
611 For each extractant, statistical significance of the difference between added and recovered
612 cyanate was tested using t -test on raw data. To analyze the effect of type of environment on

613 relative cyanate availability (i.e., $\text{NH}_4^+/\text{NCO}^-$), we used the Kruskal-Wallis test (assumption
614 for parametric procedure were not met) followed by a non-parametric multiple comparison
615 test (Dunn's test). For solving differential equations in the model, we used the "deSolve"
616 package in R (48).

617

618 **Supplementary Materials**

619 Table S1. All soil sampling sites with their location, soil pH, and cyanate, ammonium and
620 nitrate concentrations.

621

622 **References**

- 623 1. S. Jaisson, C. Pietrement, P. Gillery, Carbamylation-derived products: Bioactive
624 compounds and potential biomarkers in chronic renal failure and atherosclerosis. *Clin. Chem.* **57**, 1499–1505 (2011).
- 625 2. R. R. Dash, A. Gaur, C. Balomajumder, Cyanide in industrial wastewaters and its removal:
626 A review on biotreatment. *J. Hazard. Mater.* **163**, 1–11 (2009).
- 627 3. R. S. Kantor *et al.*, Bioreactor microbial ecosystems for thiocyanate and cyanide
628 degradation unravelled with genome-resolved metagenomics. *Environ. Microbiol.* **17**,
629 4929–4941 (2015).
- 630 4. M. Palatinszky *et al.*, Cyanate as an energy source for nitrifiers. *Nature*. **524**, 105–108
631 (2015).
- 632 5. S. Ganesh *et al.*, Single cell genomic and transcriptomic evidence for the use of alternative
633 nitrogen substrates by anammox bacteria. *ISME J.* **12**, 2706–2722 (2018).
- 634 6. N. A. Kamennaya, A. F. Post, Distribution and expression of the cyanate acquisition
635 potential among cyanobacterial populations in oligotrophic marine waters. *Limnol. Oceanogr.* **58**, 1959–1971 (2013).

638 7. K. Kitzinger *et al.*, Cyanate and urea are substrates for nitrification by Thaumarchaeota in
639 the marine environment. *Nat. Microbiol.* **4**, 234 (2019).

640 8. M. Guilloton, F. Karst, Isolation and characterization of *Escherichia coli* mutants lacking
641 inducible cyanase. *J Gen Microbiol.* **133**, 645–653 (1987).

642 9. K. Kitzinger *et al.*, Single cell analyses reveal contrasting life strategies of the two main
643 nitrifiers in the ocean. *Nat. Commun.* **11**, 1–12 (2020).

644 10. M. G. Pachiadaki *et al.*, Major role of nitrite-oxidizing bacteria in dark ocean carbon
645 fixation. *Science*. **358**, 1046–1051 (2017).

646 11. A. R. Babbin *et al.*, Multiple metabolisms constrain the anaerobic nitrite budget in the
647 Eastern Tropical South Pacific. *Global Biogeochem. Cycles*, 258–271 (2017).

648 12. A. P. Wood *et al.*, A novel pink-pigmented facultative methylotroph, *Methylobacterium*
649 *thiocyanatum* sp. nov., capable of growth on thiocyanate or cyanate as sole nitrogen
650 sources. *Arch. Microbiol.* **169**, 148–158 (1998).

651 13. A. G. Miller, G. S. Espie, Photosynthetic metabolism of cyanate by the cyanobacterium
652 *Synechococcus* UTEX 625. *Arch. Microbiol.* **162**, 151–157 (1994).

653 14. W. V. Johnson, P. M. Anderson, Bicarbonate is a recycling substrate for cyanase. *J. Biol.*
654 *Chem.* **262**, 9021–9025 (1987).

655 15. A. Taussig, The synthesis of the induced enzyme, “cyanase”, in *E. coli*. *Biochim. Biophys.*
656 *Acta*. **44**, 510–519 (1960).

657 16. N. Wybouw *et al.*, A horizontally transferred cyanase gene in the spider mite *Tetranychus*
658 *urticae* is involved in cyanate metabolism and is differentially expressed upon host plant
659 change. *Insect Biochem. Mol. Biol.* **42**, 881–889 (2012).

660 17. S. Elleuche, S. Pöggeler, A cyanase is transcriptionally regulated by arginine and involved
661 in cyanate decomposition in *Sordaria macrospora*. *Fungal Genet. Biol.* **45**, 1458–1469
662 (2008).

663 18. A. Spang *et al.*, The genome of the ammonia-oxidizing *Candidatus Nitrososphaera*
664 *gargensis*: Insights into metabolic versatility and environmental adaptations. *Environ.*
665 *Microbiol.* **14**, 3122–3145 (2012).

666 19. G. R. Stark, Reactions of cyanate with functional groups of proteins. III. Reactions with
667 amino and carboxyl Groups. *Biochemistry.* **4**, 1030–1036 (1965).

668 20. S. Malhotra, M. Pandit, J. C. Kapoor, D. K. Tyagi, Photo-oxidation of cyanide in aqueous
669 solution by the UV/H₂O₂ process. *J. Chem. Technol. Biotechnol.* **19**, 13–19 (2005).

670 21. M. P. Watts, J. W. Moreau, New insights into the genetic and metabolic diversity of
671 thiocyanate-degrading microbial consortia. *Appl. Microbiol. Biotechnol.* **100**, 1101–1108
672 (2016).

673 22. B. Widner, M. R. Mulholland, K. Mopper, Distribution, sources, and sinks of cyanate in
674 the coastal north atlantic ocean. *Environ. Sci. Technol. Lett.* **3**, 297–302 (2016).

675 23. C. Purcarea *et al.*, *Aquifex aeolicus* aspartate transcarbamoylase, an enzyme specialized for
676 the efficient utilization of unstable carbamoyl phosphate at elevated temperature. *J. Biol.*
677 *Chem.* **278**, 52924–52934 (2003).

678 24. M. Guilloton, F. Karst, Cyanate specifically inhibits arginine biosynthesis in *Escherichia*
679 *coli* K12: a case of by-product inhibition? *Microbiology.* **133**, 655–665 (1987).

680 25. P. Dirnhuber, F. Schütz, The isomeric transformation of urea into ammonium cyanate in
681 aqueous solutions. *Biochem. J.* **42**, 628–632 (1948).

682 26. IFA, in *IFA annual conference – 22–24 May 2017 Marrakech (Marocco)*. Paris: IFA
683 *International Fertilizer Association, Services PITaA* (2017; www.fertilizer.org).

684 27. B. Widner, M. R. Mulholland, K. Mopper, Chromatographic determination of nanomolar
685 cyanate concentrations in estuarine and sea waters by precolumn fluorescence
686 derivatization. *Anal. Chem.* **85**, 6661–6666 (2013).

687 28. D. A. Dzombak, F. M. M. Morel, *Surface complexation modeling: Hydrous ferric oxide*

688 (Wiley-Interscience, New York, NY, 1990).

689 29. G. R. Stark, Modification of proteins with cyanate. *Methods Enzymol.* **25**, 579–584 (1972).

690 30. T. Dashman, G. Stotzky, Adsorption and binding of amino acids on homoionic
691 montmorillonite and kaolinite. *Soil Biol. Biochem.* **14**, 447–456 (1982).

692 31. D. L. Jones *et al.*, Soil organic nitrogen mineralization across a global latitudinal gradient.
693 *Global Biogeochem. Cycles.* **23**, 1–5 (2009).

694 32. W. Wanek, M. Mooshammer, A. Blöchl, A. Hanreich, A. Richter, Determination of gross
695 rates of amino acid production and immobilization in decomposing leaf litter by a novel
696 15N isotope pool dilution technique. *Soil Biol. Biochem.* **42**, 1293–1302 (2010).

697 33. Y. Hu, Q. Zheng, S. Zhang, L. Noll, W. Wanek, Significant release and microbial
698 utilization of amino sugars and d-amino acid enantiomers from microbial cell wall
699 decomposition in soils. *Soil Biol. Biochem.* **123**, 115–125 (2018).

700 34. E. Inselsbacher *et al.*, Short-term competition between crop plants and soil microbes for
701 inorganic N fertilizer. *Soil Biol. Biochem.* **42**, 360–372 (2010).

702 35. O. Rotini, La trasformazione enzimatica dell'urea nel terreno. *Ann Fac Agric Univ Pisa.*
703 **17**, 1–25 (1956).

704 36. J. M. Bremner, M. J. Krogmeier, Evidence that the adverse effect of urea fertilizer on seed
705 germination in soil is due to ammonia formed through hydrolysis of urea by soil urease.
706 *Proc. Natl. Acad. Sci. U. S. A.* **86**, 8185–8 (1989).

707 37. M. S. Booth, J. M. Stark, E. Rastetter, Controls on nitrogen cycling in terrestrial
708 ecosystems: a synthetic analysis of literature data. *Ecol. Monogr.* **75**, 139–157 (2005).

709 38. R. E. Zdor, Bacterial cyanogenesis: impact on biotic interactions. *J. Appl. Microbiol.* **118**,
710 267–274 (2015).

711 39. J. E. Poulton, Cyanogenesis in plants. *Plant Physiol.* **94**, 401–405 (1990).

712 40. M. K. Reay *et al.*, High resolution HPLC-MS confirms overestimation of urea in soil by

713 the diacetyl monoxime (DAM) colorimetric method. *Soil Biol. Biochem.* **135**, 127–133
714 (2019).

715 41. B. Widner, C. W. Mordy, M. R. Mulholland, Cyanate distribution and uptake above and
716 within the Eastern Tropical South Pacific oxygen deficient zone. *Limnol. Oceanogr.* **63**,
717 S177–S192 (2017).

718 42. Y. Yang *et al.*, Activity and metabolic versatility of complete ammonia oxidizers in full-
719 scale wastewater treatment systems. *MBio.* **11** (2020).

720 43. E. Spasov *et al.*, High functional diversity among Nitrospira populations that dominate
721 rotating biological contactor microbial communities in a municipal wastewater treatment
722 plant. *ISME J.*, 1–16 (2020).

723 44. A. Kamyshny, H. Oduro, Z. F. Mansaray, J. Farquhar, Hydrogen cyanide accumulation and
724 transformations in non-polluted salt marsh sediments. *Aquat. geochemistry.* **19**, 97–113
725 (2013).

726 45. D. C. Wolf, H. D. Skipper, in *Methods of soil analysis, Part 2. Microbiological and*
727 *biochemical properties*, R. W. Weaver, J. S. Angle, P. S. Bottomley, Eds. (Soil Science
728 Society of America, Madison, WI, 1994), pp. 41–51.

729 46. P. Hagel, J. J. T. Gerding, W. Fieggen, H. Bloemendaal, Cyanate formation in solutions of
730 urea. I. Calculation of cyanate concentrations at different temperature and pH. *Biochim.*
731 *Biophys. Acta.* **243**, 366–373 (1971).

732 47. J. Taillades *et al.*, A pH-dependent cyanate reactivity model: application to preparative N-
733 carbamoylation of amino acids. *J. Chem. Soc. Perkin Trans. 2*, 1247–1254 (2001).

734 48. K. Soetaert, T. Petzoldt, R. W. Setzer, Package deSolve : Solving Initial Value Differential
735 Equations in R. *J. Stat. Softw.* **33**, 1–25 (2010).

736 49. D. A. Dzombak, R. S. Ghosh, G. M. Wong-Chong, *Cyanide in water and soil: Chemistry,*
737 *risk and management* (CRC press, Boca Raton, FL, 2006).

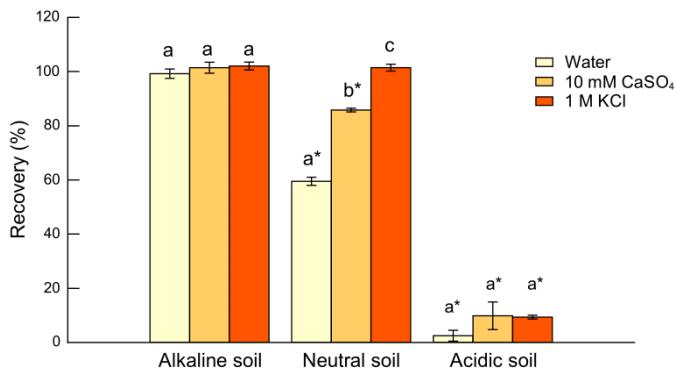
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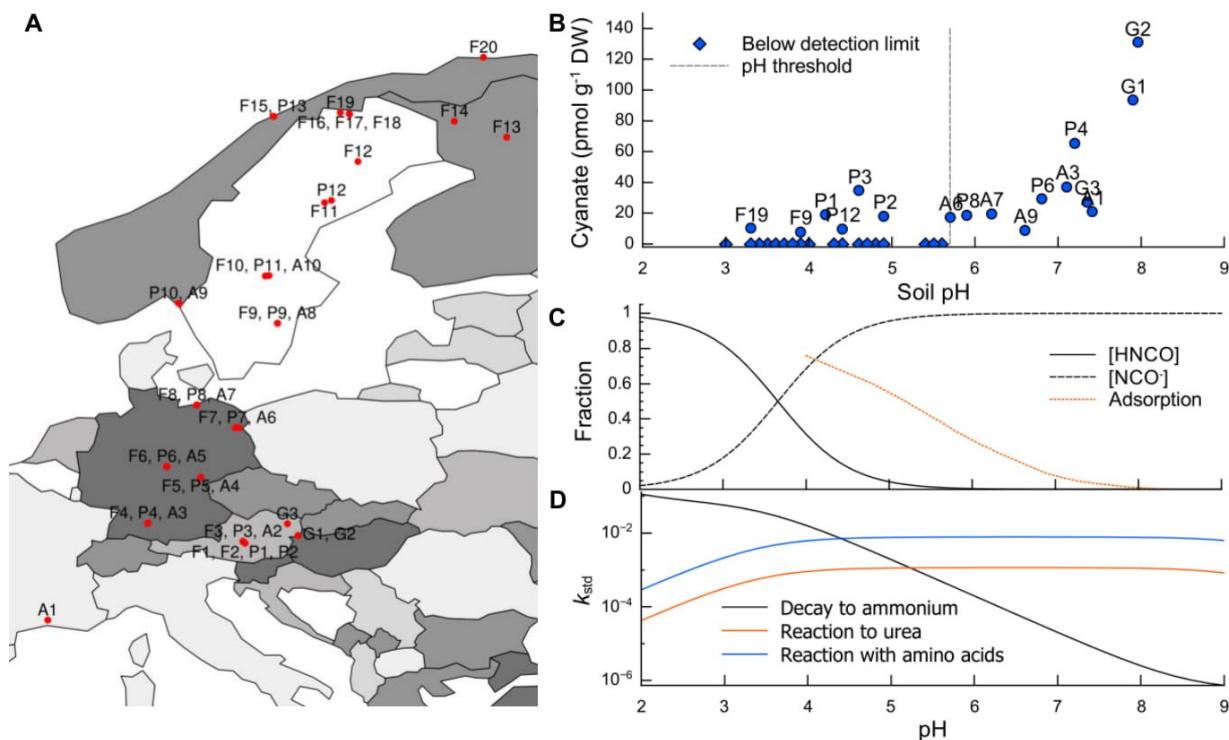
759 **Figures and Tables**



760 **Fig. 1. Comparison of extractants for determination of soil cyanate concentration.**

761 Cyanate recovery was assessed by spiking the extraction solution with potassium
762 cyanate (final concentration of 15 nM). Three extractants (water, 10 mM CaSO₄ and
763 1 M KCl) were tested for three soils: an alkaline grassland soil (soil pH = 8.3), a pH-
764 neutral mixed forest soil (soil pH = 7.0) and an acidic grassland soil (soil pH = 4.3).
765 Letters denote significant differences between extractants within each soil type (one-
766 way ANOVA followed by Tukey's HSD test). Asterisks indicate significant
767 differences between added and recovered cyanate (t-test).
768

769



770 **Fig. 2. Soil cyanate concentrations and abiotic reactions of cyanate. (A)** Map of Europe

771 displaying the 46 soil sampling sites: G, grassland; F, forest; P, pasture; A, arable.

772 **(B)** Soil cyanate concentrations (extracted using 1 M KCl) plotted as a function of

773 soil pH in 0.01 M CaCl₂. The dashed line denotes the soil pH threshold above which

774 cyanate was detectable in all soil samples. **(C)** Acid-base dependency of cyanate and

775 isocyanic acid as a function of pH ($\text{HNCO} \rightleftharpoons \text{H}^+ + \text{NCO}^-$; $pK_a = 3.66$ at 20°C). The

776 orange dotted line shows the predicted adsorption isotherm of a 10⁻⁴ M cyanate

777 solution on hydrous ferric oxide (a major component of soil influencing stabilization

778 of compounds) as a function of pH (redrawn from 49). The equilibrium surface

779 complexation constant was estimated based on correlations of acidity constants and

780 surface complexation constants fitted to adsorption data for other inorganic ions

781 (28). **(D)** Standardized rates (k_{std} ; at 20°C) of combined abiotic cyanate/isocyanic

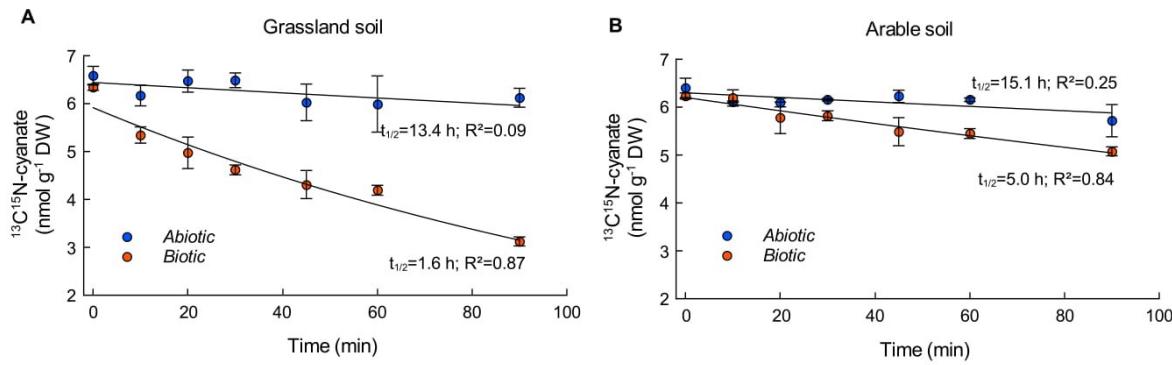
782 acid decomposition to ammonium (equations 6-8, rate constants from equations 9-

783 11), the reaction of cyanate with ammonium to urea (equation 3, rate constants from

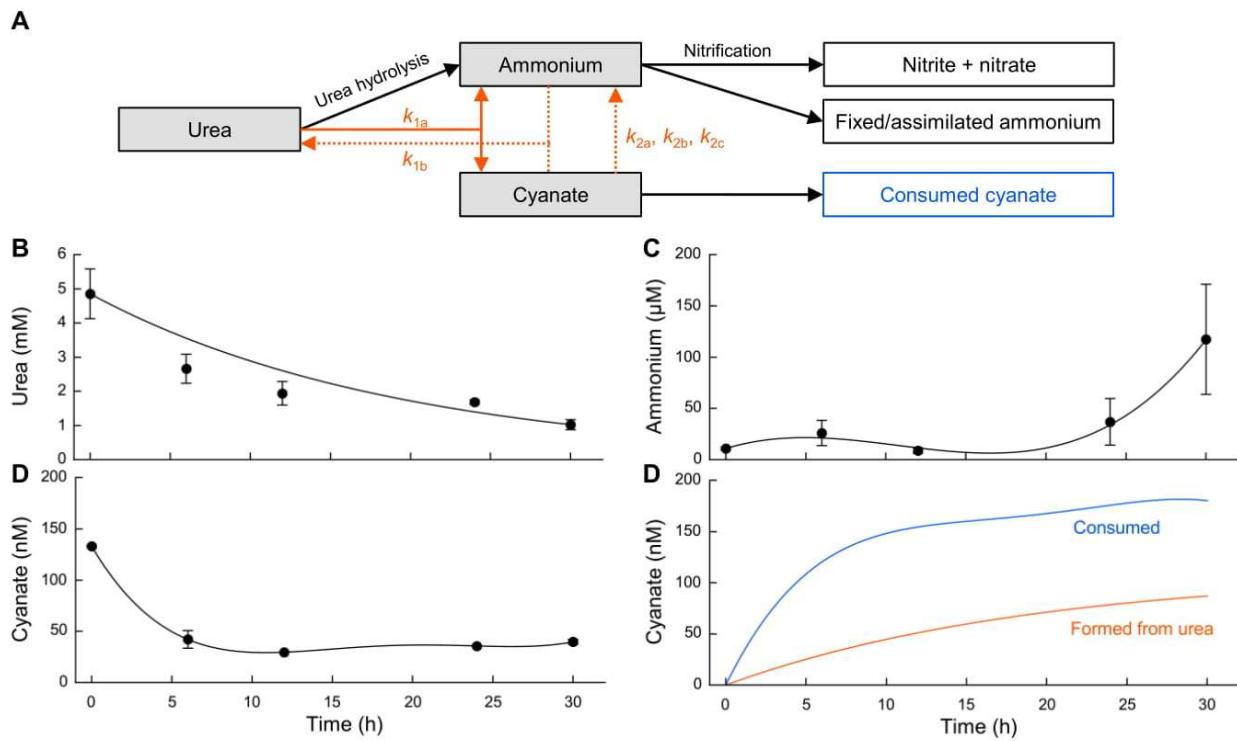
784 equation 5) and the reaction of isocyanic acid with the amino group of glycine

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786 (equation 12, rate constants from equation 13). Note that k_{std} are plotted on a
787 logarithmic scale.
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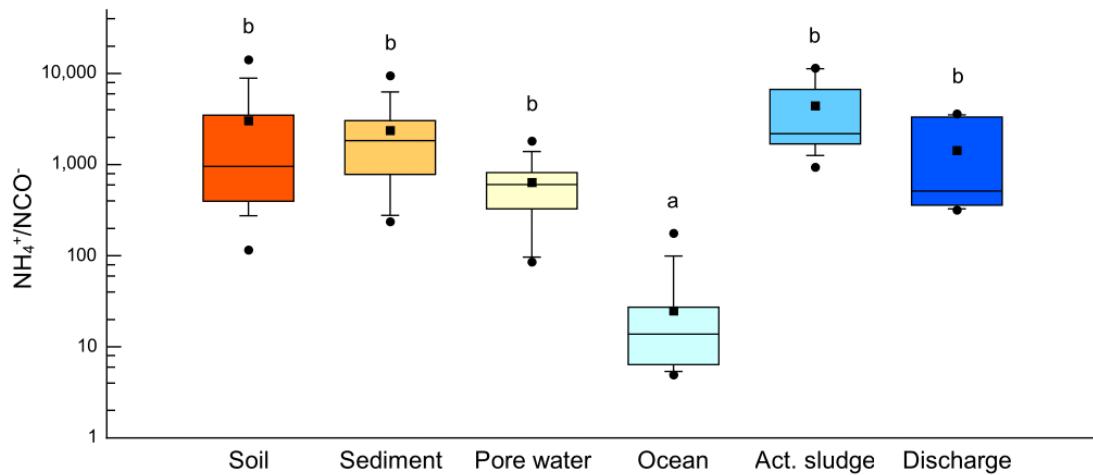


791
792 **Fig. 3.** Dynamics of soil $^{13}\text{C}^{15}\text{N}$ -cyanate consumption in two contrasting neutral soils (pH
793 = 7.4), (A) a grassland soil and (B) an arable soil (“tracer experiment”). $^{13}\text{C}^{15}\text{N}$ -
794 cyanate was added to sterile (i.e., abiotic control) and non-sterile soils, and
795 incubations were stopped after 0, 10, 20, 30, 45, 60 and 90 min. To obtain biotic
796 cyanate consumption over time, the non-sterile samples were corrected for abiotic
797 loss of cyanate derived from the sterile samples. Dynamics of cyanate consumption
798 over time for the corrected non-sterile soils and sterile soils were described by fitting
799 a first order exponential decay curve and the exponential coefficient was used to
800 calculate half-life ($t_{1/2}$) of the $^{13}\text{C}^{15}\text{N}$ -cyanate pool. Shown are average values $\pm 1\text{SE}$
801 ($n = 3$).
802



803 **Fig. 4. Gross cyanate production and consumption in soil solution of a urea-amended**

804 **arable soil (“urea addition experiment”). (A)** Schematic representation of pools
805 and fluxes used to model rates of abiotic cyanate formation from urea and microbial
806 consumption of soil cyanate. Urea, ammonium and cyanate, which are involved in
807 the chemical equilibrium reaction, are highlighted as grey boxes. Rate constants of
808 abiotic reactions are depicted in orange and were used to model cyanate fluxes based
809 on observed pool sizes. We included abiotic hydrolysis of cyanate to ammonium, as
810 the rate constants for the reaction are well established. Panels **(B-D)** show urea,
811 ammonium and cyanate concentrations in soil solution, respectively. Filled circles
812 are observed data (average \pm 1SE) at 0, 6, 12, 24 and 30 h after urea addition. **(E)**
813 Modelled rates of gross cyanate production from urea (orange line; eq. 14 using rate
814 constants from eq. 4, 5 and 9-11) are shown as cyanate accumulation over time and
815 gross cyanate consumption (blue line) calculated as the difference between cyanate
816 production and the observed net change in concentration.



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820

Fig. 5. Comparison of relative cyanate availability across different environments.

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Samples include soils (n=17), salt marsh sediments (n=12), pore water of salt marsh sediments (n=10), ocean (n=75), activated sludge (n=12) and discharge (n=9) from municipal wastewater treatment plants. Relative cyanate availability is represented as the ratio of extractable ammonium over cyanate. Different letters indicate significant differences in relative cyanate availability between environments (Kruskal-Wallis test followed by Dunn's test). The box plot shows the median (solid line within box), the average (rectangle), 25th and 75th percentiles as vertical bars, 10th and 90th percentiles as error bars and minimum and maximum as circles. Data on marine cyanate and ammonium concentrations are from Widner *et al.* (22).

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832 **Table 1. Estimates of mean residence time (MRT) of cyanate obtained from two**
833 **approaches, the urea addition and the tracer experiment.** We computed MRTs
834 of cyanate and gross cyanate production rates for 6h-time intervals of the urea
835 addition experiment. For comparative analysis of the rates, we converted them from
836 nmol L⁻¹ soil solution to rates based on a dry soil mass basis. We used MRTs to
837 calculate gross cyanate production rates for unamended soils, assuming steady-state
838 conditions, i.e., production and consumption fluxes are balanced, resulting in no
839 change in cyanate concentration (*flux = pool/MRT*).

	MRT (h)	Gross cyanate production (pmol g ⁻¹ dw d ⁻¹)
Urea addition experiment (0-30 h)		26.0
Time interval 0-6 h	3.9	39.1
Time interval 6-12 h	6.5	28.9
Time interval 12-18 h	20.9	21.1
Time interval 18-24 h	19.1	15.4
Unamended soil	5.2*	98.8‡
	24†	21.2‡
	48†	10.6‡
	72†	7.1‡

840 *Estimate from tracer addition experiment

841 †Higher MRTs assumed for conservative calculations

842 ‡Calculated using MRT assuming steady-state conditions of cyanate in soil solution

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