17β-Estradiol mineralization under field and laboratory incubations

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ABSTRACT

Mineralization studies of natural steroid hormones (e.g., 17β-estradiol, E2) are performed in environmental incubators, usually under a constant temperature such as 20°C. In this paper, we present a microcosm protocol that quantified the mineralization of E2 in soils under field temperatures. The nine agricultural soils tested had a wide range of soil organic carbon (1.1 to 5.2%) and clay (9 to 57%) contents. The calculated time over which half of the applied E2 was mineralized (E2-½) ranged from 299 to 910 d, and total E2 mineralization at 48 d (E2-TOT48) ranged from 4 to 13%. In subsequent laboratory incubations, the same soils were incubated under a constant temperature of 20°C, as well as under cyclic temperatures of 14.5°C (14 h) and 11.5°C (10h), which was within the temperature extremes observed in the field microcosms. E2-½ ranged from 157 to 686 d at 20°C and from 103 to 608 d at the cyclic temperatures, with the E2-TOT48 ranging from 6 to 21% at 20°C and from 7 to 30% under cyclic temperatures. Despite the overall 6.75 °C lower mean temperatures under the cyclic versus constant temperatures, E2 mineralization was stimulated by the temperature cycles in three soils. Regardless of the incubation, the same loamy sand soil always showed larger E2 mineralization than the other eight soils and this loamy sand soil also had the smallest E2 sorption. Current modeling approaches do not take into consideration the effects of temperature fluctuations in the field because the input parameters used to describe degradation are derived from laboratory incubations at a constant temperature. Across the eight soils, E2-½ was on average 1.7 times larger and E2-TOT48 was on average 0.8 times smaller under field temperatures than under a constant 20°C. Hence, we conclude that incubations at 20°C give a reasonable representation of

E2 mineralization occurring under field conditions to be expected in a typical Prairie summer season.

Keywords: 17β-estradiol, mineralization, temperature, fluctuations, laboratory, field

INTRODUCTION

Vertebrates excrete natural steroid hormones such as 17β-estradiol (E2) and when livestock manure or sewage sludge is applied to agricultural land, elevated concentrations of steroid hormone residues are detected in soil. [1,2] E2 can be transported from soils into the broader environment by processes such as surface runoff [3,4] and leaching. [5,6] The reported half-life of E2 in agricultural soils is 0.2 to 9.7 days. [7-9] A large portion of the initially applied E2 becomes soil-bound (non-extractable). For example as much as 56% in a silt loam, 70% in a sandy loam and 91% in a loam soil at only three days after E2 was applied. [10] However, the soil-bound fraction is slowly mineralized. [10] The smaller extractable fraction is a mixture of E2 and estrone (E1), with the ratio E2:E1 decreasing over time. E2 biodegradation to E1 has been observed in many studies, with E1 being the main or only transformation product of E2 in soils. [10-12] E1 can convert back to E2 but this has been observed only under anaerobic conditions. [13] Only a limited number of bacteria can degrade both E2 and E1, [14] and hence E2 and E1 have different degradation rates in soil. [10]

Mineralization can only be quantified by using radiolabeled [4-^{14}C] E2 in microcosm experiments, [10,11,15,16] whereby the recovery of $^{14}CO_2$ indicates that the steroid molecule has been inactivated because of ring cleavage. [17] The time that 50% of the applied E2 is mineralized (E2-½) can be calculated from these laboratory data. E2-½ has ranged from 294 to 418 d in sewage sludge, from 721 to 869 d in soil and from 2,258 to 14,146 d in biosolids. [18] The maximum E2 mineralization (E2-TOT48) is typically less than 20% of the initial E2 applied to soil microcosms, even for incubations up to 90 days. [10,19]

Temperature can affect degradation/mineralization through its influence on soil microbial activity and populations. [20,21] Mineralization studies are typically conducted in environmental chambers at a constant temperature. [7,16] Incubation studies using a range of constant temperatures have shown that E2 persistence in soil decreases with increasing temperatures, [9,10] with total E2 mineralization over a 61 day period increasing from 2% at 4 °C to 14% at 30 °C. [10] Temperature varies under field conditions and, in this paper, we present a microcosm protocol that can quantify E2 mineralization in the field. The objective of this study was to implement the in-field microcosm experiment to determine E2 parameters (E2-½, E2-TOT48) in a wide range of soils and compare these values to E2 parameters derived in the same soils under constant and cyclic temperatures in a subsequent laboratory incubation.

MATERIALS AND METHODS

Chemicals

Analytical-grade 17β-estradiol (98% chemical purity; Sigma Aldrich Chemical Company, St. Louis, MO) and 4-^{14}C labeled 17β-estradiol (99% radiochemical purity; specific activity 45 mCi/mmol; American Radiolabeled Chemicals Incorporated, St. Louis, MO) was used.

Soil Properties

Soil samples (0-10 cm) were collected from the Ap-horizons in a range of agricultural fields in the Province of Manitoba, Canada. Fields were selected based on soil maps to ensure that the soils selected had a range of soil textures and organic carbon contents. Soils were sieved (< 2 mm) and frozen at -25 ±2°C. Prior to the microcosm experiments, the moisture content of thawed soil was determined gravimetrically and distilled water was added to bring the soil to 80% of field capacity minus the liquid volume needed to add the chemical solutions. Field capacity was determined using laboratory leaching columns (11 cm in height, 2.7 cm radius) and defined as the amount of gravity retained soil moisture in these columns at 96 hours after saturation. Portions of soil were also air-dried and analyzed for a range of properties (Table 1).

Table 1. Soil characteristics and E2 sorption as determined by standard methods[1].

Soil Series	Sand (%)	Clay (%)	Silt (%)	pH	SOC (%)	N (mg/kg)	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Zn (mg/kg)	CEC (meq)	Kf ($\mu g^{1-1/n} g^{-1} ml^{1/n)}$
Denham	72	16	12	5.6	2.0	15.7	21.7	305.7	1338	294	1.9	9.9	6.96
Dezwood	57	29	15	7.0	2.3	38.2	49.3	316.3	2547	478	1.8	17.6	6.92
Halboro	85	9	6	4.4	1.1	7.2	54.0	241.0	511	106	1.9	4.1	6.12
Hibsin	65	19	17	6.8	3.3	18.5	39.3	458.3	3217	1137	3.6	26.8	8.26
Isafold	43	27	29	7.9	4.1	56.2	31.7	141.0	4768	1412	2.4	35.9	7.12
LaSalle	42	29	29	7.7	2.2	28.8	27.3	331.3	4533	519	2.9	27.8	8.47
Pembina	26	34	40	6.6	4.8	36.5	41.7	344.7	2554	385	4.7	16.8	9.00
Ramada	39	30	31	5.7	3.5	185.3	30.3	177.7	2701	632	4.2	19.2	8.67
St. Norbert	4	57	39	6.9	5.2	94.7	62.3	696.0	4494	1263	3.5	34.8	8.54

[1]Methods: %sand, silt and clay by pipette method [22]; pH (1:1) in 0.01 M $CaCl_2$ [23]; Loss of weight on ignition procedure at 360°C and 105°C [24]; NO_3-N and NH_4-N extracted with 2.0 M KCl and quantified with an automated spectrophotometric method (Maynard et al. 2008); Available phosphate was determined using the Olson ($NaHCO_3$) phosphorus test [25]; Extractable K, Ca and Mg using 1M ammonium acetate at pH 7 [26] and those measurements were used to calculate cation exchange capacity (CEC); Extractable Zn using diethylenetriaminepentaacetic acid (DTPA) [27]; Freundlich constant, Kf, determined by batch equilibrium procedures. [28] Slopes of isotherms ranged from 0.6 to 0.7.

Microcosm Experimental Design

The field experiment was set up in a Red River Clay soil on the Kelburn Farm (49° 41' N, 97° 07'W) near Winnipeg, Manitoba, Canada. The field plot (7.2 by 7.2 meters) was conducted as a randomized complete block design with five replicates (blocks) for each of the nine soil series. Soil microcosms were installed with their base 20 cm beneath the soil surface and all microcosms were covered with 5 cm of soil (Figure 1). Additional microcosms were installed with temperature probes (Thermochron® iButton, Maxim Integrated Products, San Jose, CA) to provide for in-field temperature data in selected soil types. These temperature microcosms were treated is the same way as the microcosms to which E2 solutions were applied except that the E2 solutions were replaced by water free of E2. In addition, temperature probes were installed between some microcosms at 10 and 20 cm depth below the soil surface. All probes were encased in capsules (iButton Capsules, Maxim Integrated Products, San Jose, CA) to protect them against water damage.

A subsequent laboratory experiment considered two temperature scenarios: 1) a constant temperature of 20°C, as typically used in mineralization studies, and 2) a cyclic temperature of 14.5 °C for 14 hours and of 11.5 °C for 10 hours, which was within the temperature extremes observed in the field microcosms (Figure 2).

Microcosms consisted of a 500 mL Mason jar containing a 50 mL glass beaker with 25 g soil (over-dry basis), a 20 mL scintillation vial with 8 mL of 0.5 M NaOH to trap CO_2 and a 15 mL test tube with 3 mL of acidified water (pH≤3) to preserve a humid environment without trapping CO_2. Microcosms with silica and autoclaved silica were also prepared to serve as field and laboratory controls. In both the field and laboratory experiments, soil microcosms were pre-incubated for 7 days at 20^{o}C to stimulate microbial growth and then spiked with E2 at a rate of 50 μg/kg soil and containing 1,667 Bq of [4-^{14} C] E2 per microcosm. Traps were periodically changed until cumulative $^{14}CO_2$ production had levelled off and the experiment was terminated at day 48. Scintillation cocktail (8 mL, 30% Scintisafe scintillation cocktail; Fisher Scientific, Fairlawn, NJ) was added to the removed NaOH traps and the evolved radioactivity was measured by Liquid Scintillation Counting (LSC) using an LS 6500 (Beckman Instruments, Fullerton, CA) with automated quench correction (#C Method) and a maximum counting time of 10 min.

E2 sorption

The sorption of E2 by soil was determined in triplicates using batch equilibrium procedures that followed the OECD guideline 106 [28] with soils (5 g) and E2 solutions (10 mL) rotated in glass tubes for an equilibrium time of 24 h. E2 solutions were prepared in 0.01M $CaCl_2$ at concentrations of 0.5, 1, 2, 4 and 8 mg/L and with a radioactivity of 8.30×10^5, 1.70×10^6, 3.30×10^6, 6.70×10^6 and 1.33×10^7 Bq/L, respectively. All equipment and soils were autoclaved at

121°C for 30 minutes before use as recommended by Caron et al. [29] After 24h, slurries were centrifuged for 10 minutes at 7,000 rev min^{-1} after which 1 mL sub-samples of supernatant (duplicates) were added to 8 mL scintillation vials with 5 mL 30% Scintisafe scintillation cocktail. The amount of radioactivity in solutions and experimental samples were quantified by LSC as described above. The concentration of E2 in soil was calculated by the difference between the radioactivity in the initial solution and the equilibrium solution. The Freundlich sorption coefficient, K_f [$\mu g^{1-1/n} g^{-1} mL^{1/n}$], was calculated by linear regression using the empirical Freundlich equation (log transformed): $\text{Log } C_s = \log k_f + {}^{1/n} \log C_e$, where C_s is the concentration sorbed to soil [$\mu g\ g^{-1}$] and C_e is the concentration remaining in the solution at equilibrium [$\mu g\ mL^{-1}$], and 1/n is the dimensionless Freundlich constant describing nonlinearity. Units were chosen so all isotherm lines crossed C_e=1, which is important when determining K_f. [30]

Statistical Analyses

Dissipation kinetics of E2 was generated and compared between soils using PROC NLIN in SAS version 9.4 (SAS Institute Inc., 2013). Dissipation data was described by the first-order kinetic model $C_t = C_0 e^{-kt}$, where C_t is the amount of radioactivity left in the media at time t [Bq.], C_0 is the initial amount of radioactivity in the media [Bq], and k is the first-order rate constant [d^{-1}]. Half-lives of radioactivity in media [d^{-1}] was calculated by ln2/k. The calculated half-life is equivalent to the time that 50% of the applied E2 is mineralized, which means that 50% the

steroid molecule has been inactivated because of ring cleavage. A large portion of the initially applied E2 becomes soil-bound (non-extractable), for example, between 56 and 90% of the applied E2 within 3 days. [10] However, this soil-bound fraction continues to be slowly mineralized [10] and when a compound is slowly mineralized, short-term experiments may be used to predict long-term mineralization rates using first-order kinetics. [31] Analysis of variance of total E2 mineralization data was performed using PROC GLIMMIX in SAS. The fixed effects were soil and location (field, laboratory at constant and cyclic temperature), with replicates nested within location as a random effect. The Tukey multiple comparison procedure was used for pairwise comparisons of treatment means. Treatment differences were considered significant if $P < 0.05$. PROC CORR in SAS was used to examine the associations between E2 sorption and E2-½ or E2-TOT48. All data respected normality.

RESULTS

E2 mineralization followed first-order kinetics with E2-½ ranging from 299 to 910 d in the field, from 157 to 686 d under the constant temperature regime, and from 103 to 608 d under cyclic temperatures (Table 2), in agreement with a previous laboratory incubation that observed E2-½ of up to 869 d in soil. [18] E2-TOT48 ranged from 4.3 to 10% in the field, from 5.5 to 21% under the constant temperature and from 6.7 to 30% under cyclic temperatures (Figure 3). This is within the range observed in previous laboratory incubations that report E2-TOT48 values of 2 to 35%. [10,11,18,19] Regardless of the incubation, the Halboro Loamy Sand had numerically larger

182 E2-TOT48 (Figure 3) and smaller E2-½ (Table 2) values than the other eight soils and this soil
183 had, numerically, the smallest E2 sorption (Table 1). E2 sorption in the nine soils ranged from
184 6.12 to 9.00 $\mu g^{1-1/n} g^{-1} mL^{1/n}$ and significantly limited E2-TOT48 under field (-0.59, $P = 0.001$)
185 and cyclic (-0.48; $P = 0.01$) conditions, but not under constant temperature. Sorption had no
186 significant impact on E2-½.
187
188 Incubation location had no significant impact on E2-½ values except for the following (Table 2):
189 E2-½ values significantly decreased in the order of field > constant > cyclic in the Halboro
190 Loamy Sand, in the order of field > (constant = cyclic) in the Hibsin Sandy Loam and St.
191 Norbert Clay, in the order of (field = constant) > cyclic in the Denham Sandy Loam, and in the
192 order of (field = cyclic) > (constant = cyclic) in the LaSalle Clay Loam. Also, E2-½ values were
193 significantly smaller under cyclic than constant temperatures in the Ramada Clay Loam.
194 Incubation location had no significant impact on E2-TOT48 except for the following (Figure 3):
195 E2-TOT48 significantly decreased in the order of cyclic > (field = constant) in the Denham
196 Sandy Loam and the Halboro Loamy Sand and in the order of (constant = cyclic) > field in the
197 La Salle Clay Loam and St. Norbert Clay.
198
199 Under field incubations, E2-½ was significantly smaller in Halboro Loamy Sand than all other
200 soils, except that E2-½ in Halboro Loamy Sand = Isafold Clay Loam = LaSalle Clay Loam
201 (Table 2). E-½ was also significantly smaller in Isafold Clay Loam and LaSalle Clay Loam than
202 in Hibsin Sandy Loam, but all other soil combinations had similar E2-½ values. Under the
203 constant temperature, E2-½ was significantly smaller in Halboro Loam Sand = LaSalle Clay
204 Loam = St. Norbert Clay than all other soils (Table 2). Under cyclic temperatures, E2-½

205 significantly increased in the order of Halboro Loamy Sand < St Norbert Clay = LaSalle Clay
206 Loam < LaSalle Clay Loam = Isafold Clay Loam < all other soils (Table 2).
207
208 Under field conditions, E2-TOT48 was significantly greater in the Halboro Loamy Sand than in
209 the Dezwood Sandy Clay Loam and Hisbin Sandy Loam, but all other soil combinations had
210 similar E2-TOT48 values (Figure 3). Under constant temperature, E2-TOT48 was significantly
211 greater in the Halboro Loam = LaSalle Clay Loam = St. Norbert Clay than in all other soils
212 (Figure 3). Under cyclic temperatures, there was a greater difference in E2-TOT48 among soils,
213 including that E2-TOT48 significantly decreased in the order of Halboro Loamy Sand > Denham
214 Sandy Loam = St Norbert Clay = LaSalle Clay Loam > Dezwood Sandy Clay Loam = Hisbin
215 Sandy Loam (Figure 3).
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217
218 **DISCUSSION**
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220
221 Pesticide fate models are used to estimate the transport of organic chemicals in agricultural soils,
222 including estrogens. [32,33] . Many pesticide fate models integrate the Q10 variable in equations to
223 account for the difference in degradation rate due to a 10^{o}C change in temperature. This assumes
224 that the rate of degradation depends on the activity of microorganisms because Q10 is also a
225 commonly used variable in the modeling of soil respiration. [34] By assigning a Q10 value of 2.2
226 in the pesticide fate model, the degradation rate increases by a factor of 2.2. [35] In our study, the
227 mean temperature of the cyclic incubation was 6.75 °C lower that the constant incubation at 20^{o}C

228 but soils showed either greater or similar E2 mineralization under cyclic than constant
229 incubations. Degradation is typically assumed to increase with increasing temperature and our
230 results thus suggest that that E2 mineralization was actually stimulated by the cyclic fluctuations.
231 The three soils that showed greater mineralization under cyclic than constant temperatures
232 ranged in texture from loamy sand to clay loam, and the six soils showing similar mineralization
233 under cyclic than constant temperature ranged in texture from sandy loam to clay. Hence, our
234 findings that cyclic incubations stimulate E2 mineralization appears to occur in soils regardless
235 of their soil texture and organic carbon content.
236
237 We cannot fully explain the reasons for the increase in E2 mineralization under cyclic
238 temperatures, but possible reasons include temperature impacts on bacterial growth rates, on E2
239 sorption rates, and on the rate of conversion of E2 to E1. For example, both gram-negative and
240 gram-positive bacterial cultures have been shown to grow more rapidly under cyclic than
241 constant temperatures, [36] perhaps encouraging E2 mineralization, with the effect of temperature
242 cycles on biomass activity and growth varying by bacterial species. [37] The sorption of organic
243 chemicals has been shown to decrease at cooler temperature due to a reduction in sorption
244 reaction rates, [38] and if this occurred, E2 bioavailability for mineralization could be enhanced.
245 It is also possible that the conversion from E2 to E1 was quicker in soils under constant than
246 cyclic temperatures and E1 is more slowly mineralized than E2. [9]
247
248 This is the first field experiment that used soil microcosms containing radiolabeled chemicals in
249 its experimental design (Figure 1). Lavy et al. [39] previously used radiolabeled pesticides in a
250 field experiment but their method measured long term leaching and degradation of herbicides

and not designed to measure mineralization rates. Few challenges in the design were encountered, except for a few cases of rodents nesting in the installed PVC cylinders and temporary water logging in PVC cylinders following one intense rainfall event. The median temperature of soil in microcosms was relatively similar to the median temperature measured at 10 and 20 cm depth in the Red River Clay soil in which the field plot was located (Table 3). However, the temperature probes installed outside the microcosms showed greater temperature maximums and minimums, suggesting that the microcosm jars reduced heat exchanges to some extent and this could be seen as one of the limitations of the proposed design. As shown by the summary statistics of hourly temperature data (Table 3), temperatures were relatively similar across soil types and hence we conclude that the design is appropriate for use in future studies that aim to determine mineralization parameters of a wide range of soils in a field setting.

E2 mineralization was smaller in the field than under either laboratory incubations perhaps because the cooler temperatures observed in the field, including below 10 ^{o}C early in the experiment, reduced E2 mineralization rates. In a previous study, [10] soil incubated at 10, 19 and 30 ^{o}C showed similar initial rates of E2 dissipation, but rates were significantly slower in soil incubated at 4 ^{o}C. Despite the changes in E2 parameters that occurred when exposing soils to field temperatures rather than 20^{o}C, the differences for individual soils were small. On average across soils, E2-½ values under field temperatures were 1.7 times larger than the E2-½ values under 20^{o}C incubations. To put such differences in perspective, in previous studies where we examined 2,4-D mineralization under 20^{o}C incubations, we saw this level of variation in half-lives because Ap-horizon samples were collected from different locations along a 360 m transect in a single field. [40]

Depending on the soil, E2-TOT48 values under field temperatures were 1.2 times larger to 0.3 times smaller than the E2-TOT48 values under 20^{o}C incubations (Figure 3) and, for 2,4-D mineralization, we observed similar differences in total mineralization between horizons in the same soil profile. [40] On average, E2-TOT48 values under field temperatures were only 0.8 times smaller than the E2-TOT48 values under 20^{o}C incubations.

Governments use pesticide fate models to evaluate the impact of agriculture on water resources, and to augment national water monitoring programs in the regulatory practices of pesticide environmental exposure assessments. [41] Given the many assumptions that these modeling approaches use [42] along with the spatial variability observed for the mineralization of organic chemicals in single fields and hence influence of sampling location on the value of degradation parameters, [40] we conclude that for the purpose of pesticide environmental exposure assessments, incubations at 20^{o}C give a reasonable representation of E2 mineralization under field conditions.

CONCLUSION

The type of incubation influences E2 mineralization parameters determined for soils ranging in texture from loamy sand to clay. Despite the overall 6.75 °C lower mean temperatures relative to laboratory incubations at 20 °C, cyclic temperatures of 14.5°C (14 h) and 11.5°C (10h)

297 stimulated E2 mineralization in soils. The cyclic temperatures were within the temperature
298 extremes observed in the field microcosms but, relative to both laboratory incubations, having
299 soils in microcosms in the field reduced E2 mineralization in some soils. Across the eight soils,
300 values were on average 1.7 times larger for E2-½ and 0.8 times smaller for E2-TOT48 under
301 field temperatures than under 20°C incubations. Given the many assumptions that modellers use
302 when applying pesticide fate models in the regulatory practices of pesticide environmental
303 exposure assessments and to evaluate the impact of agriculture on water resources at the national
304 scale, we conclude that for such assessments, incubations at 20^{o}C give a reasonable
305 representation of E2 mineralization under field conditions.
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308 **REFERENCES**
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FIGURE CAPTIONS

Fig 1. Field microcosm design a) view from above b) side view diagram.

Fig 2. Cyclic temperatures in the laboratory imposed on observed temperatures in field microcosms (all soils combined).

Fig 3. Total mineralization of E2 in field and in laboratory at constant and cyclic temperatures. Different letters indicate significant differences in total mineralization. Upper case letters indicate differences between location (field, laboratory at constant temperature and laboratory at fluctuating temperature). Lower case letters indicate differences among soil types within each location.

Table 2. First-order kinetic parameters of 17 β-estradiol in field and laboratory microcosms.

Soil	Model	HL	RMSE	CV	Model	HL	RMSE	CV	Model	HL	RMSE	CV
		Field microcosms				Laboratory at 20°C				Laboratory cyclic temperatures		
Denham SL	$C_t=223324e^{-0.001t}$	608Bbc	5132	2.35	$C_t=108386e^{-0.001t}$	648Bc	1926	1.81	$C_t=102485e^{-0.004t}$	166Ab	6806	7.11
Dezwood SCL	$C_t=225174e^{-0.0008t}$	891Abc	4075	1.84	$C_t=107872e^{-0.001t}$	564Abc	3654	3.46	$C_t=107663e^{-0.001t}$	608Ad	2096	1.98
Halboro LS	$C_t=216950e^{-0.002t}$	299Ca	10437	5.03	$C_t=106209e^{-0.004t}$	157Ba	6907	6.99	$C_t=99512e^{-0.007t}$	103Aa	10027	11.23
Hisbin SL	$C_t=226631e^{-0.0008t}$	910Bc	3479	1.56	$C_t=107775e^{-0.001t}$	472Abc	3197	3.04	$C_t=108309e^{-0.001t}$	537Ad	2235	2.11
Isafold CL	$C_t=220062e^{-0.002t}$	453Aab	7510	3.52	$C_t=108032e^{-0.001t}$	379Ab	3139	3.00	$C_t=105870e^{-0.002t}$	317Ac	3943	3.86
LaSalle CL	$C_t=220521e^{-0.001t}$	488Bab	8816	4.11	$C_t=102341e^{-0.004t}$	169Aa	8795	9.19	$C_t=103427e^{-0.002t}$	257ABbc	8620	8.72
Pembina CL	$C_t=224422e^{-0.001t}$	642Abc	5140	2.34	$C_t=108919e^{-0.001t}$	673Ac	2253	2.10	$C_t=107631e^{-0.001t}$	462Ad	2616	2.49
Ramada CL	$C_t=223430e^{-0.001t}$	592ABbc	5238	2.40	$C_t=108637e^{-0.001t}$	686Bc	1765	1.65	$C_t=107526e^{-0.001t}$	468Ad	2607	2.49
St. Norbert C	$C_t=228076e^{-0.001t}$	603Bbc	4747	2.13	$C_t=104451e^{-0.003t}$	203Aa	5694	5.77	$C_t=102297e^{-0.003t}$	201Ab	6152	6.37

[1]$Ct = C_0 e^{-kt}$; Ct is the radioactivity in medium (Bq) at time t (d), C_0 is the initial radioactivity in medium (Bq), and k is the first-order rate constant (d^{-1}). [2]Half-life, time it takes to reduce the initial radioactivity by 50% ($t_{1/2} = \ln 2/k$). Lower case letters within each location (field, laboratory at constant temperature or laboratory at fluctuating temperature) indicate significant differences in mineralization half-life among soils. Upper case letters indicate differences among three locations. [3]RMSE, Root Mean Square Error. [4]CV, Coefficient of Variation RMSE [CV = (RMSE/Mean) × 100].

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Table 3. Summary statistics of hourly temperature (°C) data measured in microcosm and between microcosms at 10 and 20 cm depth.

Soil	Mean	Median	Min	Max	Q1	Q3
	In microcosms installed at 20 cm depth					
Denham	12.4	12.5	9.5	15.0	11.5	13.5
Dezwood	12.4	12.5	9.5	15.0	11.5	13.5
Halboro	12.4	12.5	8.5	15.5	11.5	13.5
Isafold	12.3	12.5	9.0	15.0	11.5	13.0
LaSalle	12.1	12.0	9.0	15.0	11.5	13.0
Ramada	12.1	12.0	8.5	15.0	11.0	13.0
St. Norbert	12.7	12.5	9.5	15.0	12.0	13.5
	Between soil microcosms at 10 cm depth					
Probe 1-10	12.3	12.5	7.0	17.5	10.5	14.0
Probe 2-10	13.1	13.0	5.5	22.5	10.5	15.5
Probe 3-10	12.2	12.0	9.0	15.0	11.0	13.5
Probe 4-10	12.4	12.5	6.0	18.5	10.5	14.5
	Between soil microcosms at 20 cm depth					
Probe 1-20	13.3	13.5	10.5	16.0	12.5	14.5
Probe 2-20	12.3	12.5	10.5	14.5	11.5	13.0
Probe 3-20	12.5	12.5	9.5	15.5	11.5	13.5
Probe 4-20	13.0	13.0	8.0	18.0	11.5	14.5
Probe 5-20	12.5	12.5	10.0	15.0	11.5	13.5
Probe 6-20	12.8	12.5	10.5	15.5	12.0	13.5
Probe 7-20	12.7	12.5	10.0	15.5	12.0	13.5

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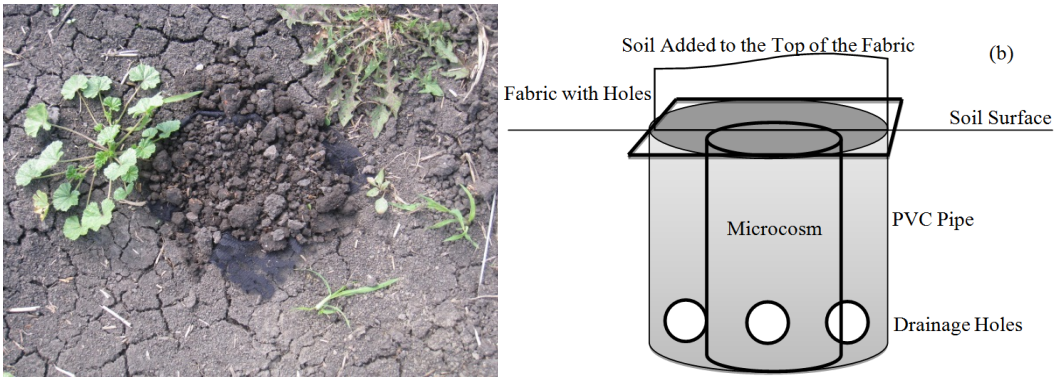

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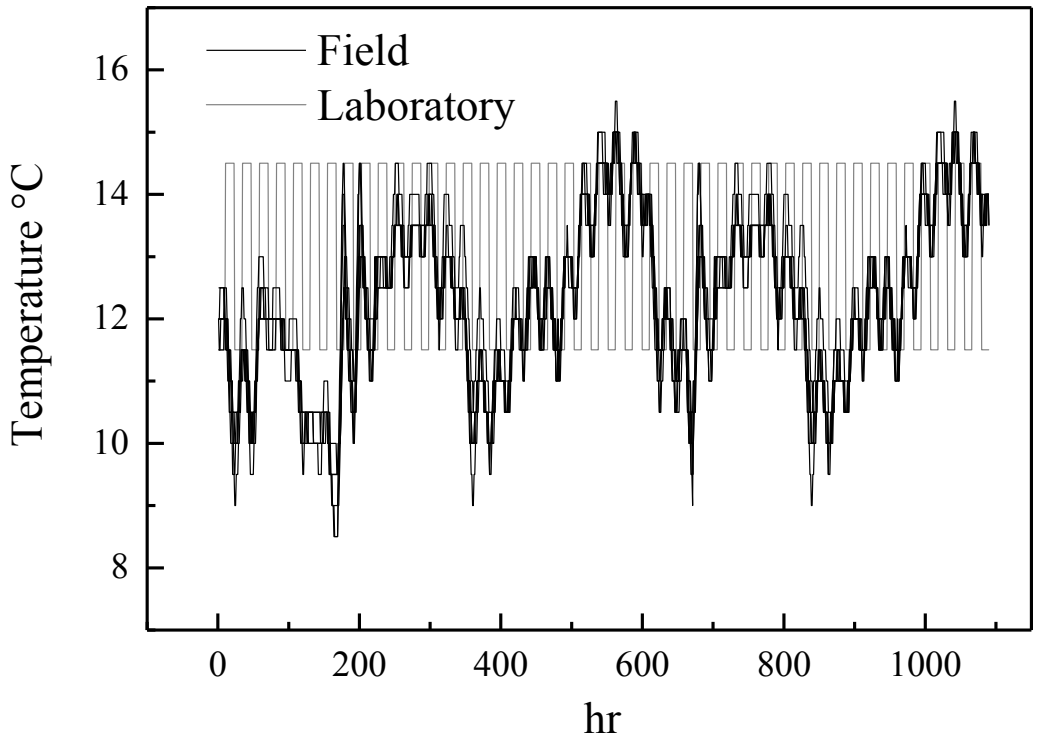

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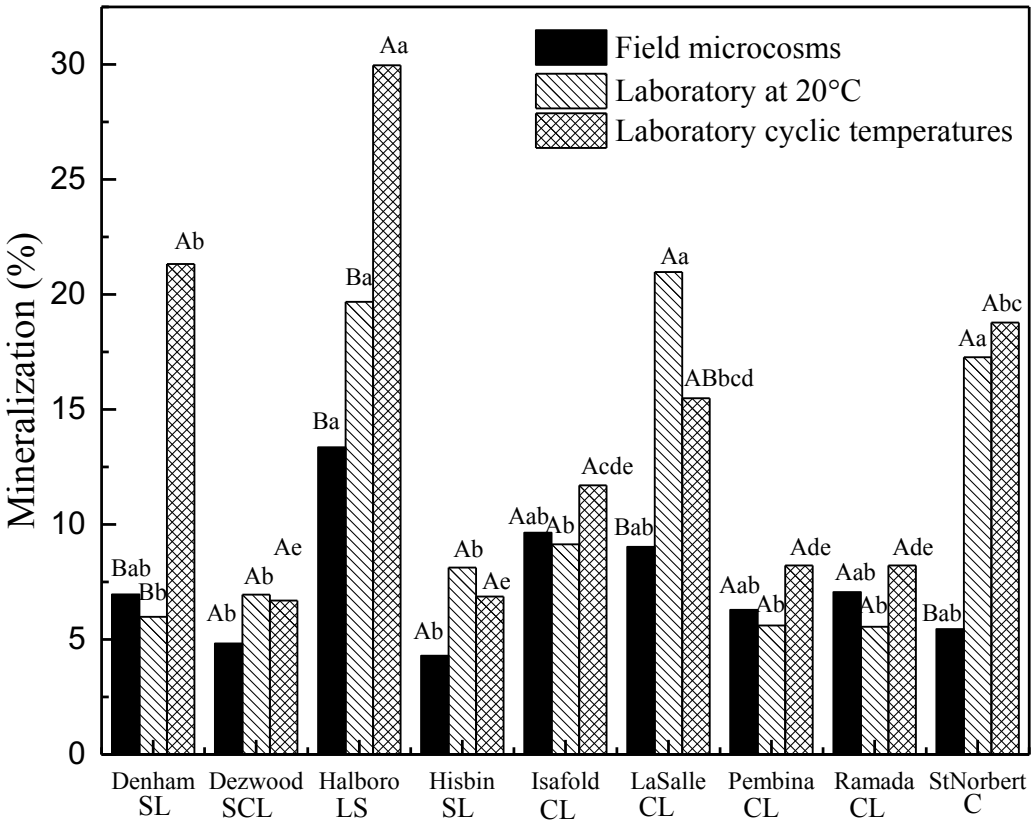

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