

1 **Nutrient depleted soil is associated with the presence of *Burkholderia pseudomallei***

2

3 Running title: Nutrient depleted soil and *Burkholderia pseudomallei*

4

5 Viriya Hantrakun¹, Patpong Rongkard¹, Malinee Oyuchua¹, Premjit Amornchai¹, Cherry Lim¹,
6 Vanaporn Wuthiekanun¹, Nicholas PJ Day^{1,2}, Sharon J. Peacock^{1,3,4}, Direk
7 Limmathurotsakul^{1,2,5,*}

8

9 ¹ Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol
10 University, Thailand

11 ² Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine,
12 Churchill Hospital, University of Oxford, United Kingdom

13 ³ Department of Medicine, Cambridge University, United Kingdom

14 ⁴ London School of Hygiene and Tropical Medicine, London, United Kingdom

15 ⁵ Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University

16

17 Corresponding author: Dr Direk Limmathurotsakul, 420/6 Mahidol-Oxford Tropical Medicine
18 Research Unit, Faculty of Tropical Medicine, Mahidol University, Rajvithee Rd, Bangkok,
19 10400 Thailand. Telephone +662-203-6304, E-mail: direk@tropmedres.ac

20

21 Word count: abstract 198, importance 147, text 3348

22 Figures: 2, Table: 1

23 Supplemental material: 1

24 **Abstract**

25 *Burkholderia pseudomallei* is a soil-dwelling bacterium and the cause of melioidosis, which kills
26 an estimated 89,000 people per year worldwide. Agricultural workers are at high risk of infection
27 due to repeated exposure. Little is known about soil physicochemical properties associated with
28 presence or absence of the organism. Here, we evaluated the soil physicochemical properties and
29 presence of *B. pseudomallei* in 6,100 soil samples collected from 61 rice fields in Thailand. The
30 presence of *B. pseudomallei* was negatively associated with the proportion of clay, proportion of
31 moisture, level of salinity, percentage of organic matter, presence of cadmium, and nutrient
32 levels (phosphorous, potassium, calcium, magnesium and iron). The presence of *B. pseudomallei*
33 was not associated with the level of soil acidity ($p=0.54$). In a multivariable logistic regression
34 model, presence of *B. pseudomallei* was negatively associated with the percentage of organic
35 matter (OR=0.06; 95%CI 0.01-0.47, $p=0.007$), level of salinity (OR=0.06; 95%CI 0.01-0.74,
36 $p=0.03$), and percentage of soil moisture (OR=0.81; 95%CI 0.66-1.00, $p=0.05$). Our study
37 suggests that in rice fields, *B. pseudomallei* thrives in those that are nutrient-depleted. Some
38 agricultural practices result in a decline in soil nutrients, which may impact on the presence and
39 amount of *B. pseudomallei* in affected areas.

40

41

42 **Importance**

43 *Burkholderia pseudomallei* is an environmental Gram-negative bacillus and the cause of
44 melioidosis. Humans acquire the disease following skin inoculation, inhalation or ingestion of
45 the bacterium in the environment. The presence of *B. pseudomallei* in soil defines geographic
46 regions where humans and livestock are at risk of melioidosis, yet little is known about soil
47 properties associated with presence of the organism. We evaluated the soil properties and
48 presence of *B. pseudomallei* in 61 rice fields in East, Central and Northeast Thailand. We
49 demonstrated that the organism was more commonly found in soils with lower levels of organic
50 matter and nutrients including phosphorus, potassium, calcium, magnesium and iron. We also
51 demonstrated that crop residue burning after harvest, which can reduce soil nutrients, was not
52 uncommon. Some agricultural practices result in a decline in soil nutrients, which may impact on
53 the presence and amount of *B. pseudomallei* in affected areas.

54

55

56 **Introduction**

57 Melioidosis, an infectious disease caused by the Gram-negative bacterium *Burkholderia*
58 *pseudomallei*, is an important global public health threat. An estimated 165,000 cases of human
59 melioidosis occur each year worldwide, of which 89,000 (54%) die (1). The disease is highly
60 endemic in Southeast Asia and Northern Australia (2), and is predicted to be endemic but is
61 grossly under-reported in many tropical and sub-tropical countries (1, 3). The crude case fatality
62 rate for melioidosis ranges from 14% to 40% and may be as high as 70% in cases given sub-
63 optimal antibiotic therapy (4-6). No licensed vaccine for melioidosis is currently available.

64

65 *B. pseudomallei* is a free-living organism found in soil and water (2), and humans acquire the
66 disease following skin inoculation, inhalation or ingestion of the bacterium in the environment
67 (7). In tropical developing countries, most patients are agricultural workers (typically rice
68 farmers) with frequent contact with soil and water. Evidence-based guidelines for the prevention
69 of melioidosis recommend that residents and visitors to melioidosis-endemic areas avoid direct
70 contact with soil and water, and wear protective gear such as boots and gloves when in direct
71 contact with soil and environmental water (7, 8). However, rubber boots are hot and make
72 walking difficult in muddy rice fields, and rubber gloves are also hot and difficult to use while
73 planting rice (9). As a result, many rice farmers continue to work in rice fields without protective
74 gear and are at high risk of melioidosis.

75

76 The presence of *B. pseudomallei* in soil defines geographic regions where humans and livestock
77 are at risk of melioidosis, but knowledge of environmental factors associated with the presence
78 of the organism in the natural setting is poor and conflicting. Laboratory studies using sterile soil

79 showed that *B. pseudomallei* grows well in soil with a high percentage of moisture (10-12), high
80 level of iron (13), optimal acidity (pH 4-8) (11, 13), and high salinity (up to 4.2 dS/m) (13). By
81 contrast, two cross-sectional studies in the natural environment in Northern Australia and
82 Northeast Thailand found that the presence of *B. pseudomallei* was negatively associated with
83 the level of iron in soil (14, 15), and a recent modelling study and an experimental field study
84 suggested that the presence of *B. pseudomallei* was not associated with soil acidity (1, 12).
85 Furthermore, both negative and positive correlations between the presence of *B. pseudomallei*
86 and soil salinity have been reported (1, 12, 15). Land use can affect the biodiversity of
87 organisms in soil (16), but there is currently no information on the association between the
88 presence of *B. pseudomallei* and agricultural practices.

89

90 Here, we report the findings of a large cross-sectional environmental survey to determine the
91 physicochemical characteristics of soil associated with the presence of *B. pseudomallei* in three
92 regions in Thailand where melioidosis is considered to be highly endemic (Northeast and East)
93 or non-endemic (Central). Our findings extend the understanding of soil properties related to
94 environmental *B. pseudomallei*.

95

96 **Materials and Methods**

97 **Study area.** East, Central and Northeast Thailand consist of 7, 21 and 20 provinces that cover
98 34,381, 93,005 and 168,854 km², and have an estimated population in 2013 of 3.9, 18.7 and 23.3
99 million, respectively (17). Northeast Thailand is a plateau surrounded by mountain ranges, and
100 most of the arable land consists of tropical sandy soil. East Thailand is characterized by short
101 mountain ranges alternating with alluvial plains. Central Thailand is a large plain consisting of
102 clay soil. Rice farming is the predominant form of agriculture in all three regions. In Thailand,
103 for administrative purposes each province is sub-divided into districts, sub-districts, communes
104 and villages. The majority of the population in all three regions live in rural settings and most
105 adults are engaged in agriculture, particularly rice farming. In 2013, land used for agriculture
106 was 57%, 48% and 60% in East, Central and Northeast Thailand, respectively (18).

107
108 To evaluate environmental factors associated with the presence of *B. pseudomallei*, we selected
109 six, seven and seven adjacent provinces in each of East, Central and Northeast Thailand,
110 respectively (Fig. 1). Three villages per province were randomly selected. Randomization was
111 performed using Stata version 14.0 (StataCorp LP, College station, Texas). Soil sampling was
112 performed in one rice field per one village. Rice fields were selected as sampling sites since rice
113 farming is a major risk factor for melioidosis (9). The sampled fields were those that had been
114 used for rice farming for at least 12 months prior to the sampling date. Written, informed
115 permission was obtained from land owners prior to sampling.

116

117 The study protocol was approved by the Ethics Committee of the Faculty of Tropical Medicine,
118 Mahidol University (MUTM 2013-021-01) and the Oxford Tropical Research Ethics Committee,
119 University of Oxford (OXTREC 1013-13).

120

121 **Soil Sampling.** Soil sampling in East, Central and Northeast Thailand was performed during the
122 dry season (from April to June) in 2013, 2014 and 2015, respectively. We used the consensus
123 guidelines for environmental sampling described by the Detection of Environmental
124 *Burkholderia pseudomallei* Working Party (DEBWorP) (19). In brief, each rice field was divided
125 into a grid system, in which 100 sampling points (10 by 10) were plotted 2.5 meters apart. At
126 each sampling point, around 30 grams of soil was removed from the base of a 30-cm hole, placed
127 in a zip bag, and kept at ambient temperature and protected from sunlight. We recorded the
128 location of sampled fields using the EpiCollect application (www.epicollect.net, Imperial
129 College, London) (20). All soil samples were processed within 48 hours of collection for the
130 identification of *B. pseudomallei* and for soil physicochemical properties.

131

132 **Identification of *B. pseudomallei*.** Ten grams of soil from each sampling point was mixed with
133 10 ml of enrichment broth consisting of threonine-basal salt solution plus colistin (TBSS-C50
134 broth) and incubated at 40°C in air for 48 hours. Ten microliters of surface liquid was then sub-
135 cultured onto Ashdown agar and incubated at 40°C in air and examined every 24 hours for 4
136 days for bacterial colonies suggestive of *B. pseudomallei*, which were initially identified on the
137 basis of colony morphotype. This included the characteristic colony morphology (purple, flat,
138 dry and wrinkled) together with six additional colony morphotypes, as described previously (21).
139 Presumptive colonies were picked from each sample and tested immediately using a specific

140 latex agglutination test for *B. pseudomallei*-specific CPS, as previously described (22). For
141 positive colonies, susceptibility to amoxicillin/clavulanic acid and arabinose assimilation were
142 determined as previously described (23). *B. pseudomallei* was defined based on the combination
143 of colony morphology, positive latex agglutination test, susceptibility to amoxicillin/clavulanic
144 acid and negative arabinose assimilation (23).

145

146 **Soil properties.** One kilogram of soil from each sampling field was made by aggregating 100
147 soil samples (10 g per each sampling point) and evaluated for four main properties, as follows.

148 (1) Physical properties: texture (proportion of sand, silt and clay) and moisture (%w/w). (2)

149 Acidity and salinity: pH, lime requirement (to adjust soil acidity; kg/100sqm) and electrical

150 conductivity (dS/m). (3) Chemical properties: total nitrogen (mg/kg), available phosphorous

151 (mg/kg), exchangeable potassium (mg/kg), exchangeable calcium (mg/kg), available magnesium

152 (mg/kg), extractable sulphur (mg/kg), total iron (g/kg), total cadmium (mg/kg), exchangeable

153 sodium (mg/kg) and cation exchange capacity (cmol/mg). (4) Biological related factors: organic

154 matter (%w/w) and carbon to nitrogen ratio (C:N ratio) (see Table S5 in the supplemental

155 material). All soil properties were evaluated by iLab Asia (Kanchanaburi, Thailand) except for

156 total iron and total cadmium which were evaluated by Central Laboratory (Bangkok, Thailand).

157 Both laboratories were registered with the Ministry of Agriculture Thailand as standardized

158 national soil testing laboratories.

159

160 **Agricultural practices.** A closed-end interviewee-based questionnaire was used to collect the

161 information about agricultural practices. For illiterate participants, the questionnaire was read to

162 the participant and completed by trained research staff in accordance with their responses.

163 Questions included fertilizer used, rice field management (before planting and after harvest) in
164 the 12 months before the sampling date.

165
166 **Sample size calculation.** To determine the optimal sample size, we performed a pilot study of
167 soil sampling in four rice fields in Chachoengsao province, East Thailand. Three of four rice
168 fields (75%) were culture positive for *B. pseudomallei*. We calculated that 60 rice fields (3 rice
169 fields per province) were needed to determine environmental factors associated with *B.*
170 *pseudomallei* with a power of 80% at an alpha error of 5%.

171
172 **Statistical analysis.** The outcomes of interest were positivity of *B. pseudomallei* in rice fields
173 and its association with soil properties. Binary and continuous variables were compared by using
174 the Fisher's exact test and Mann-Whitney test, respectively. Soil properties associated with the
175 presence of *B. pseudomallei* were evaluated using univariable and multivariable logistic
176 regression. The final multivariable logistic regression models were developed using a purposeful
177 selection method (24). Sensitivity analysis was conducted using region-stratified analysis. We
178 also used ordered logistic regression to evaluate the association between soil properties and
179 quantity of *B. pseudomallei*. The number of positive sampling points for *B. pseudomallei* within
180 a rice field was used to represent the quantity of *B. pseudomallei* distribution in the field. The
181 Spearman correlation coefficient was used to evaluate the correlation between soil properties. All
182 statistical tests were performed using Stata version 14.0 (StataCorp LP, College station, Texas).
183 The final database with the data dictionary are publicly available online
184 (<https://figshare.com/s/b44c335a9b321ab19325>).

185

186 **Results**

187 **Distribution of *B. pseudomallei* in Northeast, East and Central Thailand.** Of 6,100 soil
188 samples collected from 61 rice fields (100 soil samples per rice field), 1,046 were culture
189 positive for *B. pseudomallei* (Fig. 1). A total of 30 of 61 rice fields (49%) had at least one
190 sampling point that was culture positive for the organism. Percentages of rice fields culture-
191 positive for *B. pseudomallei* were 57% (12 of 21 rice fields), 68% (13 of 19 rice fields) and 24%
192 (5 of 21 rice fields) in Northeast, East and Central Thailand, respectively. The percentage of rice
193 fields culture-positive for *B. pseudomallei* in the Northeast and East were higher than that in
194 Central Thailand (57% vs. 24%, $p=0.06$ and 68% vs. 24%, $p=0.01$), while the percentage was not
195 significantly different between the Northeast and East (57% vs. 68%, $p=0.53$).

196

197 For the rice fields that were culture-positive for *B. pseudomallei*, the median number of positive
198 sampling points were 53 (range 2 to 98), 16 (range 1 to 81) and 1 (range 1 to 63) in Northeast,
199 East and Central Thailand, respectively (see Table S1 in the supplemental material). The median
200 number of positive sampling points in the Northeast and East were both higher than that in
201 Central Thailand ($p=0.01$ and $p=0.002$), while the number was not significantly different
202 between the East and Northeast ($p=0.61$).

203

204 **Characteristics of soil and agricultural practices.** Overall comparison of soil properties among
205 three regions showed that soil from Central Thailand had the highest median percentage of clay
206 (53%), followed by the Northeast (45%) and East (32%). Soil acidity (pH) varied considerably,
207 ranging from very acid (pH=4.9) to carbonate-rich soil (pH=8.1), but was not significantly

208 different between the three regions ($p=0.68$). Soil salinity, as determined by electrical
209 conductivity and expressed in dS/m, was very low in all fields sampled (<2.0 dS/m).

210
211 Farmers were interviewed about land management before and after rice planting (including the
212 fertilizer used, and crop residue burning before and after harvest) in the 12 months before the
213 sampling date. Of 61 rice fields evaluated, 54 (89%) were treated with chemical fertilizer, 17
214 (28%) with organic fertilizer made from plant material, 22 (36%) with organic fertilizer made
215 from animal dung, and 39 (64%) with biological fertilizer such as effective microorganisms.
216 Owners of 24 (39%) rice fields burned their fields between rice planting seasons. The median
217 percentage of organic matter in fields with a history of burning was not significantly lower than
218 that of others (0.81 vs. 0.84 %w/w, $p=0.82$).

219
220 **Association between soil physicochemical properties and *B. pseudomallei*.** We found that the
221 presence of *B. pseudomallei* was associated with nutrient-depleted soil (Fig. 2; see also Table S2
222 in the supplemental material). Presence of the organism was negatively associated with the
223 percentage of soil moisture ($p<0.001$), the level of soil salinity ($p=0.001$), presence of cadmium
224 ($p<0.001$) and levels of multiple nutrients including available phosphorous ($p=0.03$),
225 exchangeable potassium ($p<0.001$), exchangeable calcium ($p=0.001$), available magnesium
226 ($p=0.002$) and total iron ($p=0.002$). Levels of overall nutrients and total nutrient fixing capacity
227 of soil determined by organic matter and cation exchange capacity, respectively, were also
228 negatively associated with the presence of *B. pseudomallei* (both p values <0.001). The carbon to
229 nitrogen ratio, which is used to determine how easily bacteria can decompose organic material in
230 soil, was also negatively associated with the presence of *B. pseudomallei* ($p=0.01$). Presence of

231 the organism was positively associated with the proportion of sand ($p=0.02$), negatively
232 associated with the proportion of clay ($p=0.002$), and not associated with the proportion of silt
233 ($p=0.68$). Presence of *B. pseudomallei* was not associated with soil acidity ($p=0.54$), or
234 agricultural practices. Many soil physicochemical properties were strongly correlated (see Table
235 S3 in the supplemental material).

236
237 We used multivariable logistic regression analysis and found that the presence of *B.*
238 *pseudomallei* was negatively associated with the percentage of organic matter (OR=0.06; 95%CI
239 0.01-0.47, $p=0.007$), level of salinity (OR=0.06; 95%CI 0.01-0.74, $p=0.03$), and level of soil
240 moisture (OR=0.81; 95%CI 0.66-1.00, $p=0.05$) (Table 1). A sensitivity analysis was conducted
241 by including region as a stratification variable, which gave comparable results.

242
243 In addition, we also used ordered logistic regression to further evaluate the association between
244 the quantity of *B. pseudomallei* distribution in rice fields and soil physicochemical factors. We
245 observed that the number of sampling points culture positive for *B. pseudomallei* was also
246 negatively associated with the percentage of organic matter (OR=0.06; 95%CI 0.01-0.32,
247 $p=0.001$), level of soil moisture (OR=0.78; 95%CI 0.66-0.91, $p=0.002$) and level of salinity
248 (OR=0.07; 95%CI 0.01-0.53, $p=0.01$) (see Table S4 in the supplemental material).

249

250 **Discussion**

251 The results of our large environmental study demonstrated an association between the presence
252 of *B. pseudomallei* and nutrient-depleted soil in rice fields in Thailand. Negative associations
253 between the presence of *B. pseudomallei* and nutrient levels in the soil were observed for each of

254 the nutrients evaluated (with the exception of total nitrogen, exchangeable sodium and
255 extractable sulphur) and for organic matter and cation exchange capacity, which represent levels
256 of overall nutrients and total nutrient fixing capacity of soil, respectively. This is also supported
257 by the negative association between the presence of *B. pseudomallei* and the level of salinity,
258 which could also represent the level of soil nutrients in the environment (12). Our findings are
259 important because nutrients in the soil are effected by agricultural practices, and crop residue
260 burning after harvest is not uncommon in Thailand and many other tropical countries. There is
261 strong evidence to show that burning can reduce soil nutrients by eliminating crop residues and
262 soil organisms present on the soil surface (25). Poor agricultural practices could impact on the
263 presence and amount of *B. pseudomallei*. This suggests that changes in agricultural practice and
264 improvement of soil nutrient content might also be essential to reduce the distribution of *B.*
265 *pseudomallei* and incidence of melioidosis.

266
267 Our study also highlights the difference between findings from experimental soil inoculated with
268 *B. pseudomallei*, environmental studies in small areas where melioidosis is endemic, and this
269 large environmental study. For example, soil moisture was positively associated with presence of
270 *B. pseudomallei* in experimental soil studies (10-12), and environmental studies of small areas
271 where melioidosis is endemic (26, 27). It has been postulated that *B. pseudomallei* can move
272 from deeper soil layers to the surface during the rainy season and rising water table where it may
273 then multiply (28). Our study shows that soil in Northeast Thailand (where *B. pseudomallei* is
274 abundant in soil) is mostly sandy soil with a low level of organic matter and moisture, while soil
275 in Central Thailand (where *B. pseudomallei* is less abundant), is mostly clay soil with high level

276 of organic matter and moisture. This is also supported by a recent finding of the presence of *B.*
277 *pseudomallei* in a desert region outside the wet tropics in Northern Australia (29).

278

279 Organic matter in soil contains vital nutrients and influences the diversity and biological activity
280 of soil organisms (25). The negative association between soil organic matter and the presence of
281 *B. pseudomallei* is consistent with two previous environmental studies in Northern Australia (15)
282 and Northeast Thailand (30), which showed that the level of organic carbon was negatively
283 associated with presence of *B. pseudomallei*. The level of organic carbon is a measure of the
284 carbon contained within the soil organic matter. It is possible that soils with high organic matter
285 have high biotic stress because abundant soil microorganisms are competing for substrates, water
286 or growth factors (31), which may inhibit the survival or growth of *B. pseudomallei*. This is
287 supported by an environmental study showing that low microbial density in soil is associated
288 with the presence of *B. pseudomallei* (27, 32) and that *Bacillus amyloliquefaciens* extracted from
289 soil samples can inhibit the growth of *B. pseudomallei* (32). It is also possible that depletion of
290 individual nutrients such as iron supports the growth of *B. pseudomallei*, which has a range of
291 mechanisms to persist in low iron environments (33). An additional possibility is that
292 environmental stress selects for persister cells of *B. pseudomallei*, as has been recently shown for
293 *Pseudomonas aeruginosa* in nutrient-limited conditions and in biofilm (34). *B. pseudomallei* are
294 taken up by amoebae, which in vitro are associated with survival in the presence of disinfecting
295 agents and antimicrobial drugs (35, 36), and may represent an additional survival advantage for
296 *B. pseudomallei* in nutrient-depleted soil.

297

298 Our findings suggest that extremely low levels of salinity (such as <0.1 dS/m) may be an indirect
299 measure of nutrient depletion in rice fields. This is because soil salinity as estimated by
300 measuring electrical conductivity represents soluble salts of soil nutrients, including sodium,
301 chloride, magnesium, calcium, potassium and nitrate. Our finding is consistent with an
302 experimental study in Northern Australia (12), which showed that *B. pseudomallei* grew well in
303 soil with low electrical conductivity (0.1 dS/m) but could not survive in commercial soil, which
304 has a high level of organic based compost and high electrical conductivity (0.7 dS/m). Although
305 a recent modelling study proposed a positive association between salinity level and presence of
306 *B. pseudomallei*, this estimation was based on soil salinity for all land (undisturbed land,
307 agricultural land, sports fields, etc) with an electrical conductivity ranging from 0 to >20 dS/m
308 (1). It is also possible that the effect of salinity in rice fields may be different from non-rice
309 fields. For example, rice fields may be intentionally flooded and drained repeatedly to reduce
310 salinity to a very low level (<2.0 dS/m) (37), and this could lead to the loss of water-soluble
311 nutrients from the soil (38-40).

312
313 *B. pseudomallei* can survive well in soil under laboratory condition with pH ranging from 4 to 8
314 (13), and our study supports the lack of association between presence of *B. pseudomallei* and pH.

315
316 A limitation of our study is that soil sampling was only performed during the dry season over a
317 period of three years. We chose to sample during the dry season to control for variation in the
318 presence of *B. pseudomallei* and soil physicochemical properties associated with seasonal
319 changes. Recent environmental studies showed that soil properties were not different between
320 the dry and wet season (14), and that changes in the presence of *B. pseudomallei* in the soil with

321 very low salinity level (<2.0 dS/m) measured over three years were minimal (12). It is possible
322 that the presence of *B. pseudomallei* in rice fields would have been generally higher if the study
323 was conducted during the rainy season. Although the difference in percentage of organic matter
324 between fields with and without a history of burning was not observed in our study, this could be
325 because of the cross-sectional study design or other confounding factors. For example, some
326 fields were burned more than 12 months before the study was conducted.

327

328 In summary, our large cross-sectional environmental survey has shown that the presence of the
329 important human pathogen *B. pseudomallei* is associated with nutrient-depleted rice fields.
330 Further investigations are required to evaluate whether changes in agricultural practices could
331 effectively enhance soil nutrients, and whether these could reduce the distribution of *B.*
332 *pseudomallei* in rice fields.

333

334

335 **Acknowledgements**

336 We thank the farmers, village heads and soil sampling volunteers who participated in the study.

337 We thank Weerawat Wongasa, Sayan Langla, Sittikorn Rongsumlee, Taveewat

338 Boonyakamolset, Prapass Wannapinij, Prapaporn Srilohasin, Boonkoed Siriphong and Piyamas

339 Buakaew for their laboratory and administrative support. The authors declare no conflict of

340 interests.

341

342 **Funding information**

343 The study was funded by the National Institute of Allergy and Infectious Diseases (Y1-AI-4906-

344 09). DL is supported by an Intermediate Fellowship awarded by the Wellcome Trust

345 (101103/Z/13/Z). The funders had no role in study design, data collection and interpretation, or

346 the decision to submit the work for publication.

347

348 **References**

349

- 350 1. **Limmathurotsakul D, Golding N, Dance DAB, Messina JP, Pigott DM, Moyes CL,**
351 **Rolim DB, Bertherat E, Day NPJ, Peacock SJ, Hay SI.** 2016. Predicted global
352 distribution of *Burkholderia pseudomallei* and burden of melioidosis. Nat Microbiol
353 **1**:15008. <http://dx.doi.org/10.1038/nmicrobiol.2015.8>
- 354 2. **Wiersinga WJ, Currie BJ, Peacock SJ.** 2012. Melioidosis. N Engl J Med **367**:1035-
355 1044. <http://dx.doi.org/10.1056/NEJMra1204699>
- 356 3. **Currie BJ, Dance DA, Cheng AC.** 2008. The global distribution of *Burkholderia*
357 *pseudomallei* and melioidosis: an update. Trans R Soc Trop Med Hyg **102 Suppl 1**:S1-4.
358 [http://dx.doi.org/10.1016/S0035-9203\(08\)70002-6](http://dx.doi.org/10.1016/S0035-9203(08)70002-6)
- 359 4. **Chierakul W, Anunnatsiri S, Short JM, Maharjan B, Mootsikapun P, Simpson AJ,**
360 **Limmathurotsakul D, Cheng AC, Stepniewska K, Newton PN, Chaowagul W, White**
361 **NJ, Peacock SJ, Day NP, Chetchotisakd P.** 2005. Two randomized controlled trials of
362 ceftazidime alone versus ceftazidime in combination with trimethoprim-
363 sulfamethoxazole for the treatment of severe melioidosis. Clin Infect Dis **41**:1105-1113.
364 <http://dx.doi.org/10.1086/444456>
- 365 5. **Limmathurotsakul D, Wongratanacheewin S, Teerawattanasook N, Wongsuvan G,**
366 **Chaisuksant S, Chetchotisakd P, Chaowagul W, Day NP, Peacock SJ.** 2010.
367 Increasing incidence of human melioidosis in Northeast Thailand. Am J Trop Med Hyg
368 **82**:1113-1117. <http://dx.doi.org/10.4269/ajtmh.2010.10-0038>

- 369 6. **White NJ, Dance DA, Chaowagul W, Wattanagoon Y, Wuthiekanun V,**
370 **Pitakwatchara N.** 1989. Halving of mortality of severe melioidosis by ceftazidime.
371 *Lancet* **2**:697-701.
- 372 7. **Limmathurotsakul D, Kanoksil M, Wuthiekanun V, Kitphati R, deStavola B, Day**
373 **NP, Peacock SJ.** 2013. Activities of daily living associated with acquisition of
374 melioidosis in northeast Thailand: a matched case-control study. *PLoS Negl Trop Dis*
375 **7**:e2072. <http://dx.doi.org/10.1371/journal.pntd.0002072>
- 376 8. **Faa AG, Holt PJ.** 2002. Melioidosis in the Torres Strait islands of far North Queensland.
377 *Commun Dis Intell Q Rep* **26**:279-283.
- 378 9. **Suntornsut P, Wongsuwan N, Malasit M, Kitphati R, Michie S, Peacock SJ,**
379 **Limmathurotsakul D.** 2016. Barriers and Recommended Interventions to Prevent
380 Melioidosis in Northeast Thailand: A Focus Group Study Using the Behaviour Change
381 Wheel. *PLoS Negl Trop Dis* **10**:e0004823.
382 <http://dx.doi.org/10.1371/journal.pntd.0004823>
- 383 10. **Tong S, Yang S, Lu Z, He W.** 1996. Laboratory investigation of ecological factors
384 influencing the environmental presence of *Burkholderia pseudomallei*. *Microbiol*
385 *Immunol* **40**:451-453.
- 386 11. **Chen YS, Chen SC, Kao CM, Chen YL.** 2003. Effects of soil pH, temperature and
387 water content on the growth of *Burkholderia pseudomallei*. *Folia Microbiol (Praha)*
388 **48**:253-256.
- 389 12. **Kaestli M, Harrington G, Mayo M, Chatfield MD, Harrington I, Hill A,**
390 **Munksgaard N, Gibb K, Currie BJ.** 2015. What drives the occurrence of the

- 391 melioidosis bacterium *Burkholderia pseudomallei* in domestic gardens? PLoS Negl Trop
392 Dis **9**:e0003635. <http://dx.doi.org/10.1371/journal.pntd.0003635>
- 393 13. **Wang-Ngarm S, Chareonsudjai S, Chareonsudjai P.** 2014. Physicochemical factors
394 affecting the growth of *Burkholderia pseudomallei* in soil microcosm. Am J Trop Med
395 Hyg **90**:480-485. <http://dx.doi.org/10.4269/ajtmh.13-0446>
- 396 14. **Thanapat S, Supunnipa W, Pisit C, Rasana SW, Sorujisiri C.** 2013. Seasonal variation
397 of soil environmental characteristics affect the presence of *Burkholderia pseudomallei* in
398 Khon Kaen, Thailand. Afr J Microbiol Res **7**:6.
399 <http://dx.doi.org/10.5897/AJMR2012.2335>
- 400 15. **Baker AL, Ezzahir J, Gardiner C, Shipton W, Warner JM.** 2015. Environmental
401 Attributes Influencing the Distribution of *Burkholderia pseudomallei* in Northern
402 Australia. PLoS One **10**:e0138953. <http://dx.doi.org/10.1371/journal.pone.0138953>
- 403 16. **Newbold T, Hudson LN, Hill SLL, Contu S, Lysenko I, Senior RA, Börger L,
404 Bennett DJ, Choimes A, Collen B, Day J, De Palma A, Díaz S, Echeverria-Londoño
405 S, Edgar MJ, Feldman A, Garon M, Harrison MLK, Alhousseini T, Ingram DJ,
406 Itescu Y, Kattge J, Kemp V, Kirkpatrick L, Kleyer M, Correia DLP, Martin CD,
407 Meiri S, Novosolov M, Pan Y, Phillips HRP, Purves DW, Robinson A, Simpson J,
408 Tuck SL, Weiher E, White HJ, Ewers RM, Mace GM, Scharlemann JPW, Purvis A.**
409 2015. Global effects of land use on local terrestrial biodiversity. Nature **520**:6.
410 <http://dx.doi.org/10.1038/nature14324>
- 411 17. **The Bureau of registration Administration, Ministry of Interior, Thailand.** 2013.
412 Thailand Demographic, 25 February 2014 ed.
413 http://stat.bora.dopa.go.th/stat/y_stat56.html Accessed 23 October 2015

- 414 18. **Office of Agricultural Economics, Ministry of Agriculture and Cooperatives,**
415 **Thailand.** 2013. Lands used for agriculture census 2013, 21 January 2015 ed, Office of
416 Agricultural Economics.
417 http://www.oae.go.th/download/use_soilNew/soiNew/landused2556.html Accessed date
418 25 November 2015
- 419 19. **Limmathurotsakul D, Dance DA, Wuthiekanun V, Kaestli M, Mayo M, Warner J,**
420 **Wagner DM, Tuanyok A, Wertheim H, Yoke Cheng T, Mukhopadhyay C,**
421 **Puthucheary S, Day NP, Steinmetz I, Currie BJ, Peacock SJ.** 2013. Systematic review
422 and consensus guidelines for environmental sampling of *Burkholderia pseudomallei*.
423 PLoS Negl Trop Dis 7:e2105. <http://dx.doi.org/10.1371/journal.pntd.0002105>
- 424 20. **Aanensen DM, Huntley DM, Feil EJ, al-Own F, Spratt BG.** 2009. EpiCollect: linking
425 smartphones to web applications for epidemiology, ecology and community data
426 collection. PLoS One 4:e6968. <http://dx.doi.org/10.12688/f1000research.4702.1>
- 427 21. **Chantratita N, Wuthiekanun V, Boonbumrung K, Tiyawisutsri R, Vesaratchavest**
428 **M, Limmathurotsakul D, Chierakul W, Wongratanacheewin S, Pukritiyakamee S,**
429 **White NJ, Day NP, Peacock SJ.** 2007. Biological relevance of colony morphology and
430 phenotypic switching by *Burkholderia pseudomallei*. J Bacteriol 189:807-817.
431 <http://dx.doi.org/10.1128/JB.01258-06>
- 432 22. **Smith MD, Wuthiekanun V, Walsh AL, Pitt TL.** 1993. Latex agglutination test for
433 identification of *Pseudomonas pseudomallei*. J Clin Pathol 46:374-375.
- 434 23. **Wuthiekanun V, Smith MD, Dance DA, Walsh AL, Pitt TL, White NJ.** 1996.
435 Biochemical characteristics of clinical and environmental isolates of *Burkholderia*
436 *pseudomallei*. J Med Microbiol 45:408-412.

- 437 24. **Bursac Z, Gauss CH, Williams DK, Hosmer DW.** 2008. Purposeful selection of
438 variables in logistic regression. *Source Code Biol Med* **3**:17.
439 <http://dx.doi.org/10.1186/1751-0473-3-17>
- 440 25. **Food and Agriculture Organization of the United Nations.** 2005. The importance of
441 soil organic matter. <http://www.fao.org/3/a-a0100e.pdf> Accessed date 5 November 2015
- 442 26. **Kaestli M, Mayo M, Harrington G, Ward L, Watt F, Hill JV, Cheng AC, Currie BJ.**
443 2009. Landscape changes influence the occurrence of the melioidosis bacterium
444 *Burkholderia pseudomallei* in soil in northern Australia. *PLoS Negl Trop Dis* **3**:e364.
445 <http://dx.doi.org/10.1371/journal.pntd.0000364>
- 446 27. **Serm Swan RW, Royros P, Khakhum N, Wongratanacheewin S, Tuanyok A.** 2015.
447 Direct detection of *Burkholderia pseudomallei* and biological factors in soil. *Trans R Soc*
448 *Trop Med Hyg* **109**:462-468. <http://dx.doi.org/10.1093/trstmh/trv040>
- 449 28. **Thomas AD, Forbes-Faulkner J, Parker M.** 1979. Isolation of *Pseudomonas*
450 *pseudomallei* from clay layers at defined depths. *Am J Epidemiol* **110**:515-521.
- 451 29. **Yip TW, Hewagama S, Mayo M, Price EP, Sarovich DS, Bastian I, Baird RW,**
452 **Spratt BG, Currie BJ.** 2015. Endemic melioidosis in residents of desert region after
453 atypically intense rainfall in central Australia, 2011. *Emerg Infect Dis* **21**:1038-1040.
454 <http://dx.doi.org/10.3201/eid2106.141908>
- 455 30. **Ngamsang R, Potisap C, Boonmee A, Lawongsa P, Chaianunporn T,**
456 **Wongratanacheewin S, Rodrigues JL, Serm Swan RW.** 2015. The Contribution of Soil
457 Physicochemical Properties to the Presence and Genetic Diversity of *Burkholderia*
458 *pseudomallei*. *Southeast Asian J Trop Med Public Health* **46**:38-50.

- 459 31. **Pepper IL, Gerba CP, Gentry T, Maier RM.** 2009. Environmental Microbiology,
460 Second ed. Academic Press, California.
- 461 32. **Potisap C, Boottanun P, Boonmee A, Rodrigues HLM, Sermswan RW.** 2016.
462 Identification of *Bacillus amyloliquefaciens* in soil with compounds that kill
463 *Burkholderia pseudomallei*, Abstract in: Proceedings of 8th World Melioidosis Congress;
464 2016 August 8–10; Cebu, Phillippines.
- 465 33. **Ribolzi O, Rochelle-Newall E, Dittrich S, Auda Y, Newton PN, Rattanavong S,**
466 **Knappik M, Soulileuth B, Sengtaheuanghong O, Dance DA, Pierret A.** 2016. Land
467 use and soil type determine the presence of the pathogen *Burkholderia pseudomallei* in
468 tropical rivers. Environ Sci Pollut Res Int <http://dx.doi.org/10.1007/s11356-015-5943-z>
- 469 34. **Nguyen D, Joshi-Datar A, Lepine F, Bauerle E, Olakanmi O, Beer K, McKay G,**
470 **Sihnel R, Schafhauser J, Wang Y, Britigan BE, Singh PK.** 2011. Active starvation
471 responses mediate antibiotic tolerance in biofilms and nutrient-limited bacteria. Science
472 **334**:982-986. <http://dx.doi.org/10.1126/science.1211037>
- 473 35. **Inglis TJ, Rodrigues F, Rigby P, Norton R, Currie BJ.** 2004. Comparison of the
474 susceptibilities of *Burkholderia pseudomallei* to meropenem and ceftazidime by
475 conventional and intracellular methods. Antimicrob Agents Chemother **48**:2999-3005.
476 <http://dx.doi.org/10.1128/AAC.48.8.2999-3005.2004>
- 477 36. **Howard K, Inglis TJ.** 2005. Disinfection of *Burkholderia pseudomallei* in potable water.
478 Water Res **39**:1085-1092. <http://dx.doi.org/10.1016/j.watres.2004.12.028>
- 479 37. **Inthong S, Vityakon P, Sriboonlue V, Trelo-ges V.** 2005. Characteristics of nutrient
480 leaching in a sandy soil of northeast Thailand. <http://agris.fao.org/agris->
481 [search/search.do?recordID=TH2006000046](http://agris.fao.org/agris-search/search.do?recordID=TH2006000046). Accessed 27 August 2016.

- 482 38. **Stoate C, Boatman ND, Borralho RJ, Carvalho CR, de Snoo GR, Eden P.** 2001.
483 Ecological impacts of arable intensification in Europe. *J Environ Manage* **63**:337-365.
- 484 39. **Pathak BK, Kazama F, Toshiaki I.** 2004. Monitoring of Nitrogen Leaching from a
485 Tropical Paddy in Thailand. *Agricultural Engineering International: the CIGR Journal of*
486 *Scientific Research and Development* **VI**.
487 <http://www.cigrjournal.org/index.php/Ejournal/article/view/545/539>
- 488 40. **Food and Agriculture Organization of the United Nations.** Saline soil and their
489 management. <http://www.fao.org/docrep/x5871e/x5871e04.htm>. Accessed 28 June 2016.
- 490 41. **Tukey JW.** 1977. *Exploratory Data Analysis*, First ed. Pearson, Washington.
- 491
492

493 **Figure legends**

494 **FIG 1 Distribution of *B. pseudomallei* in Central, East and Northeast Thailand.**

495 (a) Map of Thailand. (b) Location of the 61 rice fields evaluated. Red and white circles, culture
496 positive and negative for *B. pseudomallei*, respectively. Province codes represent Phetchabun
497 (C1), Phitsanulok (C2), Pathum Thani (C3), Saraburi (C4), Lopburi (C5), Nakhon Nayok (C6)
498 and Bangkok (C7) in Central Thailand, Chachoengsao (E1), Prachinburi (E2), Sa Kaeo (E3),
499 Chanthaburi (E4), Chonburi (E5) and Rayong (E6) in the East, and Burirum (NE1), Chaiyaphum
500 (NE2), Khon Kaen (NE3), Udon Thani (NE4), Nong Bua Lam Phu (NE5), Loei (NE6) and
501 Nakhon Ratchasima (NE7) in the Northeast.

502

503 **FIG 2 Soil physicochemical properties associated with the presence of *B. pseudomallei***

504 Box–whisker plots indicate median, interquartile range and distribution of the data. Dots indicate

505 the outliers (data located outside 1.5 times of interquartile range) (41). Red and grey boxes

506 represent rice fields culture positive (Pos) and negative (Neg) for *B. pseudomallei*, respectively.

507 * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ and NS=Not Significant.

508

509

510 **Tables**

511

512 **TABLE 1 Soil physicochemical properties associated with the presence of *B. pseudomallei***
513 **in a multivariable logistic regression model**

514

Soil physicochemical characteristics	Adjusted odds ratio (95% CI)	p value
Organic matter (%w/w)	0.06 (0.01-0.47)	0.007
Electrical conductivity (dS/m)	0.06 (0.01-0.74)	0.03
Moisture (%)	0.81 (0.66-1.00)	0.05

515



