

1 Carbon-driven eco-agriculture without nitrogen deficiency

2
3 *Masato Oda, Livestock & Environment, Japan International Research Center for
4 Agricultural Sciences, Tsukuba, Japan

5
6 Kenji Tamura, Graduate School of Life and Environmental Sciences, University of Tsukuba,
7 Tsukuba, Japan

8
9 Hiroko Nakatsuka, Graduate School of Life and Environmental Sciences, University of
10 Tsukuba, Tsukuba, Japan

11
12 Miki Nakata, Department of Local Produce and Food Sciences, University of Yamanashi,
13 Yamanashi, Japan

14
15 Yukimi Hayashi, Sitio TKM, Sao Paulo, Brazil

16
17 *Corresponding author: 1-1 Ohwashi, Tsukuba, 305-8686, +81-298-38-6362,
18 ODA.Masato@affrc.go.jp

19 20 21 **Abstract**

22
23 A farmer grew crops by adding only organic material with a high C:N ratio (40) to the soil for
24 30 years. He focused on the role of carbon in increasing the number of microorganisms. This
25 idea was based on the concepts of 1) indirect crop management via microorganisms and 2)
26 providing carbon to microorganisms for energy. Here, we name this practice “carbon -driven
27 eco-agriculture” (CDEA). We determined the effect of CDEA on a laterite soil vegetable field
28 in Sao Paulo for 4 years. The yield exceeded the national average. Soil aggregates formed to
29 29 cm thickness, and the microbial activity was one order of magnitude higher than that in a
30 conventional control field. The output/input ratios of carbon and nitrogen were 1.88 –2.35 and
31 3.58–6.00, respectively, indicating a sustainable system for these elements. Incorporating
32 high-C:N-ratio (>20) organic material results in nitrogen deficiency. However, our results
33 indicate that large numbers of microorganisms provide crops with sufficient nitrogen at low
34 concentrations. This method overcomes the yield limitation of chemical fertilizer application
35 and reverses soil degradation.

36 37 38 **Introduction**

39 Scientific fertilization was initiated by Thaer in which organic matter was used as fertilizer
40 that doubled agricultural productivity. After Liebig discovered that plants absorb inorganic
41 nutrition, fertilization practices changed from use of organic matter to use of chemical
42 fertilizer, and agricultural productivity improved further. The element absorbed by plants in
43 large amounts for their nutrition is nitrogen. However, Liebig was aware of the unstable
44 effects of nitrogen application, and it was later clarified that the instability arises from
45 microbial activity. Microorganisms are receiving increasing attention in agricultural contexts.

46
47 Hitoshi Mine, a farmer in Sao Paulo, Brazil, considered of growing crops by adding only
48 organic material with a high C:N (approximately 40) ratio after observing forest ecosystems.
49 He used this method for 30 years in his vegetable fields. He says, “Plants have evolved with a
50 nutrient balance produced by microorganisms. Therefore, the nutrient balance produced by

1 microorganisms is ideal for plants.” The points of his idea are 1) indirect crop management
2 via microorganisms and 2) providing carbon to microorganisms for their energy source. The
3 first idea expresses what we term as “soil improvement,” and the second idea provides us
4 with a new viewpoint on organic material application. It is consumption that is essential and
5 not the content in soil. Here, we designate this type of agriculture as “carbon-driven eco-
6 agriculture” (CDEA). Instead, of direct fertilization, balanced nutrients are supplied to crops
7 by microorganisms. We conducted a field experiment with CDEA to characterize productivity,
8 sustainability, and principles.

11 **Materials & Methods**

13 We practiced CDEA for 4 years and collected yield information. We compared the field with
14 a control field with respect to soil profile, soil total nitrogen, carbon, and free ATP on Nov 19,
15 2012. The details are as follows.

17 **Study field**

18 We determined the effect of CDEA with the cooperation of Mr. and Ms. Nakamura, vegetable
19 farmers in the city of Suzano, São Paulo. Their farm is located in a hilly area of typical laterite
20 soil. The field had deteriorated with over 40 years of fertilizer use. CDEA was initiated in
21 July 2008 and was practiced on all 2 ha of their farm by 2010. In a soil survey plot, we grew
22 lettuce (12 crops) and cabbage (2 crops), and butter cabbages planted on April 9, 2012 were
23 growing at the time of survey. We chose a neighboring farmer’s field as the control field. In
24 the control field, cassava was harvested in January 2012, and corn was grown after addition of
25 waste mushroom bed without fertilizer and harvested in July 2012. The control field was then
26 kept fallow.

28 **The specific method of CDEA**

29 The specific method was as follows: (1) the same crop was planted without a break after a
30 harvest, so that a crop was always growing; (2) approximately 15–20 t ha⁻¹ crop⁻¹ of fresh
31 waste mushroom bed (C:N ratio, 39; moisture, 61.80%; total carbon, 19.10%; and total
32 nitrogen, 0.49%) was added to approximately 10 cm of surface soil using a rotary tiller; (3) no
33 other materials (N, P, or K fertilizer, minerals, microelements, growth promoters, pH control
34 chemicals, or agricultural chemicals) were used during the period; (4) commercially available
35 seedlings and seeds were used; (5) weeds were cut with a brush cutter when they began to
36 compete with crops and were left on fields; and (6) irrigation was not applied. However,
37 under severe drought conditions, irrigation was applied the day before seeding or planting of
38 seedlings and during the following two days. We chose fresh waste mushroom bed as the high
39 C:N ratio organic material for the experiment because its properties were stable. The material
40 was transported directly from a mushroom farm (Sitio TKM, Suzano) to the field to maintain
41 its quality.

43 **Calculation of vegetable yield**

44 We summed the number of each vegetable item harvested from years 2010 to 2012 and
45 multiplied each number by the item’s standard weight. Conventional vegetable yields were
46 calculated by weight using the top five items; lettuce (46%), cabbage (23%), napa cabbage
47 (7%), radish (5%), and cauliflower (4%). The Japanese average annual yields per ha for these
48 crops are 21, 32, 32, 29, and 14 tons respectively (e-Stat 2013). We converted the weight
49 percentage to an area percentage and then multiplied this by the average yields. For example,
50 when the area of lettuce was 46%, that of cabbage was 15% (= 23% × 21/32). We calculated

1 the total yield by multiplying the area of each crop by the Japanese annual average for the
2 same crop. It was 17.6 tons ($46\% \times 21 + 15\% \times 32 + 4\% \times 32 + 4\% \times 29 + 6\% \times 14$). The
3 total area of these top five crops was 74%, so that the conventional yield per ha was 23.7 tons
4 ($=17.6 \times 100/74$).

6 **Nitrate nitrogen concentration** (determined on Dec 12, 2010)

7 Soil was sampled from the 0–10-cm soil layer. The sample was well mixed, and a 100 g
8 subsample was taken. Water was added until saturation with gentle stirring, and the mixture
9 was allowed to settle for 6 h. Nitrate was determined with test strips (Macherey-Nagel, Düren,
10 quantofix MN91313). The NO_3^- was converted to $\text{NO}_3^- \text{N}$ by multiplying by 0.2259. The rest
11 of the sample was weighed, air dried, and weighed again to determine moisture content.

13 **Soil profiles and the soil property analysis**

14 Soil profiles were described according to the Handbook of Soil Survey (Japanese Society of
15 Pedology, 1997). We collected wet soil samples from each soil layer and sieved them to 2
16 mm. We took two sets of 20-mL samples: one to determine free ATP and the other to
17 determine moisture content and carbon and nitrogen contents.

19 **Free ATP**

20 We placed samples in cups, added 50 mL of water, and stirred for 1 min with vibration (Nippi
21 inc, Tokyo, Power masher). We then added 6 mL of the surface water to a sample tube and
22 centrifuged it at 6500 rpm ($2200 \times g$) for 1 min. We then placed 100 μL of the solution using
23 an autopipette into ATP Water Test Devices (Hygiena International, Aquasnap AQ100F). We
24 mixed luciferase with the solution and measured with a luminometer (Hygiena International,
25 Camarillo, SystemSURE Plus) 20 s after mixing the luciferase. The amount of free ATP was
26 calculated using the weight and soil moisture of a paired sample.

28 **Total carbon and nitrogen analysis**

29 Paired soil samples were air dried. Total N and C contents of the soils were determined with a
30 NC analyzer (Sumitomo Chemical, Tokyo, SUMI- GRAPH NC 200F).

32 **Carbon and nitrogen exploitation by harvested product**

33 We used the Japanese average nutrition balance data per area
34 (http://www.niaes.affrc.go.jp/techdoc/dotoku/hozen_news033.pdf). We used butter cabbage
35 for the cabbage data. The values per m^2 were 28.4 C g and 3.03 N g for lettuce and 171.6 C g
36 and 14.21 N g for cabbage.

39 **Results**

41 **Crop growth and productivity**

42 Good crop growth in the treatment field (TF) with no nitrogen deficiency or pests was
43 observed (Figure 1). Irrigation was not needed even when drought period exceeded 65 days.
44 We sold 33 items, including leafy, fruit, and root vegetables. Lettuce and cabbage accounted
45 for 46% and 23% of the weight, respectively. The total annual average yield from 2010 to
46 2012 was 56.5 t ha^{-1} , which was higher than the 23.7 t ha^{-1} of the estimated Japanese average
47 conventional yield. For reference, the Japanese country average unit yield of all vegetables
48 (23.3 t ha^{-1}) is almost double that of Brazil (12.8 t ha^{-1} ; FAOSTAT, 2013).



Figure 1. Lettuce in a “carbon driven eco-agriculture” field

Fifty-four days after planting (Nov 24, 2012) at Suzano, São Paulo. Approximately 15–20 t ha⁻¹ crop⁻¹ of fresh waste mushroom bed (C:N ratio, 39; moisture, 61.80%; total carbon, 19.10%; total nitrogen, 0.49%) was added to approximately 10 cm of surface soil using a rotary tiller. There was no irrigation even when drought period exceeded 65 days. No disease or insect pests were observed (Photograph taken by M. Oda).

Soil changes

The NO₃⁻N concentration in the top 0–80 cm of soil was 5.6 mg kg⁻¹ soil, lower than the 20 mg kg⁻¹ soil lower limit (Fox et al. 1989; Breschini & Hartz 2002) for fertilizer agriculture. The structure of the soil showed that aggregates of up to 29 cm formed in the TF (Table 1, Figure 2). These aggregates were not earthworm feces but subangular blocky structures formed around plant roots. The total C in the TF was 6,520 g C m⁻², higher than that in the control field (CF). Soil carbon has been shown to increase in natural forest by approximately 0.2–12.0 g C m⁻² year⁻¹ and by 2 g C m⁻² year⁻¹ in tropical rain forest (Schlesinger 1990). In general, nitrogen input increases the net primary production but barely increases soil carbon (Paustian et al. 1990; Ogle et al. 2005). However, we found that using only high-carbon-ratio organic material drastically enhances the formation of the soil A horizon without additional nitrogen. The pores of the A horizon were large, and the bulk densities of horizons Ap1 (0.68) and Ap2 (0.84) were far smaller than that of the Bw1 horizon (1.03). These pores can absorb approximately 80 mm of rainfall. This excellent soil physical property makes farming without irrigation possible (Parikh & James 2012). It may also help in preventing soil erosion.

1

Table 1. T-C & T-N of soil layer

Field	Structure		Thickness (cm)		Bulk density		Concent (mg g ⁻¹ soil)				Amount (g m ⁻²)				C/N	
	TF	CF	TF	CF	TF	CF	T-C		T-N		T-C		T-N		TF	CF
							TF	CF	TF	CF	TF	CF	TF	CF	TF	CF
Layer 1	Ap1	Ap1	15	9	0.68	0.90	76.6	27.2	4.66	2.05	7,812	1,665	475	166	16.4	13.3
Layer 2	Ap2	Ap2	14	13	0.84	0.95	54.1	27.1	3.52	1.99	6,357	2,962	413	246	15.4	13.6
Layer 3	AB	A3	12	12	0.94	0.97	21.0	22.1	1.42	1.51	2,357	2,480	159	175	14.8	14.7
Layer 4	Bw1	Bw1	13	16	1.03	0.99	14.3	18.6	0.83	1.13	1,919	3,070	111	179	17.3	16.5
Layer 5	Bw2	Bw2	26	22	1.00	0.92	13.0	15.6	0.63	0.82	3,354	3,409	162	166	20.7	19.0
Layer 6	Bw3	Bw3	20	28	0.96	0.85	12.2	15.0	0.53	0.61	2,337	4,029	102	145	23.0	24.6
Total	—	—	100	100	—	—	—	—	—	—	24,135	17,616	1,422	1,077	—	—
T-F											6,520		345			

2

3

TF: treatment field. CF: control field. Bw1: Bw1 horizon of treatment field.

4

The TF was provided with approximately 15–20 t ha⁻¹ crop⁻¹ of waste mushroom bed in 15 applications from Jul 2008 to Nov 2012. The CF (control field) was left fallow after corn harvest in July 2012.

5

6

7



8

9

Figure 2. Landscapes and the soil sections

10

a. Treatment field; 40 years of fertilizing agriculture was converted in July 2008, and 15 crops (12 lettuce crops, 2 cabbage crops, and 1 butter cabbage) were planted without a break after a harvest, so that a crop was always growing; b. Control field; a neighboring farmer's fallow field (corn was harvested 4 months previously and cassava 10 months previously, and waste mushroom bed was used for the first time for the corn; Photographs taken by H. Nakatsuka).

11

12

13

14

1

Table 2. Yield ratio of carbon and nitrogen for 15 crops

	All layers				Top three layers			
	T-C (g m^{-2})		T-N (g m^{-2})		T-C (g m^{-2})		T-N (g m^{-2})	
Base	CF	Bw1	CF	Bw1	CF	Bw1	CF	Bw1
Original	17,616	13,137	1,077	761	7,107	4,756	587	275
Present	24,135	24,135	1,422	1,422	16,525	16,525	1,047	1,047
Output	6,520	10,999	345	661	9,418	11,769	461	772
Products	856	856	79	79	856	856	79	79
Net output	7,375	11,854	424	740	10,274	12,625	540	851
Input	5,014	5,014	129	129	5,014	5,014	129	129
Net output/input	1.47	2.36	3.30	5.76	2.05	2.52	4.20	6.62
Output/input	1.30	2.19	2.68	5.14	1.88	2.35	3.58	6.00

2

3 CF: control field. Bw1: Bw1 horizon of treatment field.

4 T-C and T-N changes in the treatment field were estimated in the CF and the Bw1 base.

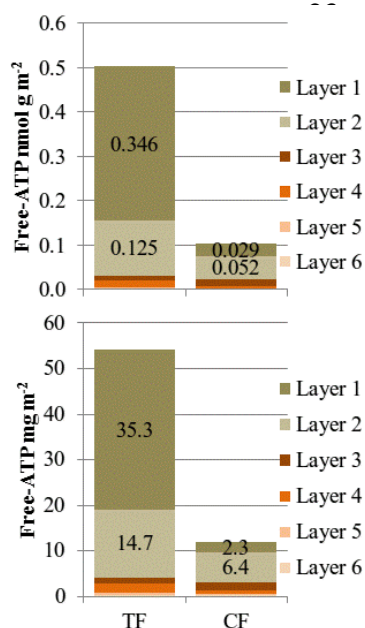
5 "Present" denotes the present value from the treatment field. "Output" denotes the difference
6 between the present levels and the original levels. "Input" denotes the total amount of waste
7 mushroom bed.

8

Output/input ratio of T-C and T-N

9 The total nitrogen of the TF increased by 345 g m^{-2} compared with CF over the whole soil
10 profile (Table 2). The net increase was 424 g when 79 g of nitrogen removed in the harvest
11 was included. The total nitrogen input from the waste mushroom bed was 129 g , so that the
12 net output/input (O/I) ratio was 3.30. This value estimates the lower limit because the CF had
13 already received one input of waste mushroom bed, and the fallow period restores soil fertility
14 (Szott et al. 1999). If all the soil of the TF was the same as the Bw1 horizon, the O/I ratio was
15 5.76 (Table 2). This value estimates the upper limit because nitrogen removal from the field
16 by crop harvest must be excluded. Thus the practical O/I ratio will be 2.68–5.14. If the O/I
17 ratio is >0 , it is sustainable. If it is >1 , it is sustainable without external input. Thus, nitrogen
18 input is no longer necessary. The O/I ratio of carbon was from 1.30 to 2.19, meaning carbon
19 input is also no longer necessary to sustain the current productivity. When we use the above
20 estimates with only the top three soil layers, the O/I ratio increases further (nitrogen, 3.58–
21 6.00; carbon, 1.88–2.35).

22

**Table 3. Free ATP of soil layer**

Field	Content (nmol g^{-1} soil)			Amount (mg m^{-2})		
	TF	CF	TF/CF	TF	CF	TF/CF
Layer 1	0.346	0.029	11.9	35.3	2.3	15.0
Layer 2	0.125	0.052	2.4	14.7	6.4	2.3
Layer 3	0.012	0.017	0.7	1.3	2.0	0.7
Layer 4	0.015	0.003	4.4	2.1	0.5	3.7
Layer 5	0.002	0.002	0.8	0.5	0.5	1.0
Layer 6	0.002	0.001	2.4	0.3	0.2	1.9
Total	0.502	0.104	4.8	54.2	11.9	4.6

TF: treatment field. CF: control field.

Figure 3. Free ATP in soil

TF: treatment field, CF: conventional field, Layer: refer to
Table 1. Amounts of free ATP were calculated by multiplying
the concentration of each soil layer by the soil weight.

1 **Microbial activity**

2 Microbial activity is very high in the topsoil (Table 3, Figure 3). This activity is associated
3 with the carbon and nitrogen concentrations, indicating that the soil carbon and nitrogen are
4 largely microbial. The microbial activity of CDEA was one order of magnitude higher than
5 that in the conventional field.

8 **Discussion**

10 **Principle**

11 CDEA contradicts the general understanding of soil management. The lower limit of available
12 nitrogen in soil is considered to be 20 NO₃⁻N mg kg⁻¹ soil as nitrate (Fox et al. 1989;
13 Breschini & Hartz 2002). The principle is the same in organic farming (Entz et al. 2001).
14 When organic material with a high C:N ratio is added to the soil, soil microorganisms use the
15 carbon as a substrate and multiply simultaneously using the available soil nitrogen thereby
16 decreasing the level in the soil (Blair & Prince 1928). To prevent this depletion, materials
17 with a low C:N ratio of <20 are used to suppress the growth of microorganisms in soil
18 (Carbon-nitrogen relationships 2013). These are what we call composts. Thus, as long as a
19 high available nitrogen level in the soil is maintained, the growth of microorganisms is
20 suppressed. Adding organic material with a high C:N ratio to the soil without composting is
21 the point of CDEA. For preventing nitrogen deficiency, a large number of microorganisms are
22 needed. In fact, the microbial activity of CDEA was one order of magnitude higher than that
23 in the control field. Recent studies of an intensive wheat crop rotation in Australia reported
24 that free-living microorganisms fixed 20 kg ha⁻¹ year⁻¹ of nitrogen, 30%–50% of the amount
25 required for the cultivation system (Gupta & Paterson 2006). It appears feasible to produce
26 the necessary amount of nitrogen by doubling the number of microorganisms. These results
27 support the idea of indirect crop management via microorganisms.

29 **Productivity and sustainability**

30 Organic farming rarely exceeds conventional farming in yield levels (Seufert et al. 2012), but
31 the productivity of CDEA is four times the national average. In addition, it was achieved
32 without plant protection. Conventional farming may achieve the same level. However, the
33 higher soil nitrate levels will necessitate plant protection necessary. With respect to
34 sustainability, CDEA required approximately 5000 g m⁻² of total carbon input; however, the
35 high productivity of CDEA provides considerable carbon to the soil. The O/I ratio of carbon
36 is greater than 1 at present, meaning that CDEA is sustainable without external input. Further
37 studies are required to elucidate the details of the changes in the O/I ratio of carbon and
38 whether CDEA is useful for other field crops such as sugarcane, corn, wheat, rice, etc.

41 **Conclusions**

- 42 1. Carbon-driven eco-agriculture (CDEA) is an agricultural method that incorporates high-C:
43 N-ratio organic matter into soil for providing carbon to microorganisms. It aims at indirect
44 crop management via microorganisms.
- 45 2. It achieved a four-fold national average productivity in a 2 ha vegetable field.
- 46 3. Soil NO₃⁻N concentration was lower than the standard limit for conventional agriculture,
47 but crop growth was vigorous and nitrogen deficiency was not observed.
- 48 4. The aggregates formed up to 29 cm thickness in 2.5 years.
- 49 5. The output/input ratio of soil total nitrogen and carbon were estimated as 2.68 to 6.00 and
50 1.30 to 2.35, respectively.

- 1 6. Microbial activities were one order of magnitude higher than that in a fallow field.
2 7. CDEA is based on high microbial activities.
3 8. CDEA is sustainable for C and N because these output/input ratios are larger than 1.
4 9. Further studies of CDEA's productivity and sustainability are needed.

5 **Acknowledgments**

6 We thank T. Nakamura and M. Nakamura for conducting the field experiment. We thank H.
7 Mine, I. Nakamura, A. Fukushima, K. Toriyama, S. A. Ephraim, and J. S. Caldwell for
8 discussion and their advice. We thank S. Nakamura and M. Yonemura for assisting in soil
9 analysis. The authors would like to thank Enago (www.enago.jp) for the English language review.

10 **References**

- 11
12
13
14
15 Fox RH, Roth GW, Iversen KV, Piekielek, WP. 1989. Soil and tissue nitrate tests compared
16 for predicting soil nitrogen availability to corn. *Agron J* 81 : 971–974
17 <https://www.agronomy.org/publications/aj/abstracts/81/6/AJ0810060971>
18 Breschini SJ, Hartz TK. 2002. Presidedress soil nitrate testing reduces nitrogen fertilizer use
19 and nitrate leaching hazard in lettuce production. *HortScience* 37 : 1061–1064
20 <http://hortsci.ashspublications.org/content/37/7/1061.full.pdf>
21 Entz MH, Guilford R, Gulden R. 2001. Crop yield and soil nutrient status on 14 organic farms
22 in the eastern portion of the northern Great Plains. *Can J Plant Sci* 81: 351–354
23 <http://pubs.aic.ca/doi/pdf/10.4141/P00-089>
24 Blair AW, Prince AL. 1928. The Influence of Heavy Applications of Dry Organic Matter on
25 Crop Yields and on the Nitrate Content of the Soil. *Soil Sci* 25: 281–288
26 http://journals.lww.com/soilsci/Citation/1928/04000/The_Influence_of_Heavy_Applications_of_Dry_Organic.4.aspx
27 Carbon-nitrogen relationships. 2013. Washington state university. *Carbon-nitrogen*
28 *relationships*. http://whatcom.wsu.edu/ag/compost/fundamentals/needs_carbon_nitrogen.
29 (Accessed 2 Jul 2013)
30 Guputa V, Paterson J. 2006. Free-living bacteria lift soil nitrogen supply. *Farming Ahead*
31 169: 40
32 http://www.csiro.au/en/Outcomes/Food-and-Agriculture/~/_Media/CSIROau/Files/PDF/FA_FEB06_bacteria_Ento_PDF%20Standard.pdf
33 Seufert V, Ramankutty N, Norman J, Foley JA. 2012. Comparing the yields of organic and
34 conventional agriculture. *Nature* 485: 229–234. doi:10.1038/nature11069.
35 <http://www.nature.com/nature/journal/v485/n7397/full/nature11069.html>
36 FAOSTAT. 2013. *Food and Agriculture Organization of the United Nations*.
37 <http://faostat.fao.org>. (Accessed 2 Jul 2013)
38 Schlesinger WH. 1990. Evidence from chronosequence studies for a low carbon-storage
39 potential of soils. *Nature* 348: 232–234
40 <http://alliance.la.asu.edu/temporary/students/Phil/Schlesinger.pdf>
41 Paustian K, Andren O, Clarholm M, Hansson AC, Johansson G, Lagerlof J, Lindberg T,
42 Pettersson R, Sohlenius B. 1990. Carbon and nitrogen budgets of four agro-ecosystems with
43 annual and perennial crops, with and without N fertilization. *J Appl Ecol* 27: 60–84
44 <http://www.jstor.org/stable/2403568>
45 Ogle SM, Breidt FJ, Paustian K. 2005. Agricultural management impacts on soil organic
46 carbon storage under moist and dry climatic conditions of temperate and tropical regions.
47 *Biogeochemistry* 72: 87–121. doi:10.1007/s10566-004-0360-2.
48 <http://www.jstor.org/stable/20055160>
49
50

- 1 Parikh SJ, James BR. 2012. Soil: The Foundation of Agriculture. *Nature Education* 3(10.: 2
2 [http://www.nature.com/scitable/knowledge/library/soil-the-foundation-of-agriculture-](http://www.nature.com/scitable/knowledge/library/soil-the-foundation-of-agriculture-84224268)
3 [84224268](http://www.nature.com/scitable/knowledge/library/soil-the-foundation-of-agriculture-84224268)
4 Szott LT, Palm CA, Buresh RJ. 1999. Ecosystem fertility and fallow function in the humid
5 and subhumid tropics. *Agroforestry Systems* 47: 163–196.
6 <http://link.springer.com/article/10.1023%2FA%3A1006215430432?LI=true#page-1>
7
8 e-Stat. 2013. *Portal site of official statistics of Japan*. <http://e-stat.go.jp>. (Accessed 2 Jul
9 2013)