Effects of biogas slurry fertilization on fruit economic traits and soil nutrients of *Camellia oleifera* Abel

Lu You¹,², Shuqin Yu¹,², Huiyun Liu¹,², Chutian Wang¹,², Zengliang Zhou¹,², Ling Zhang¹,², Dongnan Hu¹,²*

¹ College of Forestry, Jiangxi Agricultural University, Nanchang, Jiangxi, China, ² Jiangxi Provincial Key Laboratory of Silviculture, Jiangxi Agricultural University, Nanchang, China

* dnhu98@163.com

**Abstract**

*Camellia oleifera* Abel (*C. oleifera*) absorb nutrients from surrounding soils and its yield is highly influenced by these nutrients and by fertilizer application. Thus, the soil nutrients play a central role in *C. oleifera* production. This study investigated the effects of biogas slurry applications on soil nutrients and economic traits of *C. oleifera* fruits. Five different amounts of biogas slurry (0, 10, 20, 30, or 40 kg/plant/year, three applications per year) were used as fertilizer for *C. oleifera* plants in 2015 and 2016. The nutrients of rhizosphere soil and the economic traits, including fruit yield, seed rate, and oil yield of *C. oleifera* fruit, were measured each year. The results showed that fertilization with biogas slurry significantly increased soil organic matter, available nitrogen (N), phosphorus (P), and potassium (K) both in 2015 and 2016. Increases in soil available N, P, and K were maximal in the highest slurry application group followed by the second highest application group. The oil yield correlated with the content of soil available P in both 2015 and 2016, and with soil organic matter in 2015. Fertilization with biogas slurry decreased the saturated fatty acid content in fruit but had no effect on the unsaturated fatty acid content. In conclusion, fertilization with biogas slurry increased rhizosphere soil nutrients and fruit economic traits of *C. oleifera* and rates of at least 30 kg/plant/year had the most positive effects. This study expands the knowledge of fertilization with biogas slurry in *C. oleifera* production.

**Introduction**

Biogas slurry is a secondary product produced by anaerobic fermentation of bio-materials, which has been widely used as a fertilizer in agricultural production. Biogas slurry is not only an environmentally friendly organic fertilizer, but also an efficiently utilized waste material. Recently, livestock excrements, such as feces and urine, have become a severe problem in China. These challenge many animal premises and create extensive environmental pollution [1]. Anaerobic fermentation is one of the most effective solutions for this challenge. The main product of anaerobic fermentation is biogas, which is an important and clean energy. The by-
product, biogas slurry can be used in agricultural and forestry production [2, 3]. Currently, the use of biogas slurry as a fertilizer has drastically increased in China and many other Asian countries, not only due to the considerable cost of chemical fertilizers, but also to utilize the high nutrient level in biogas slurry [4, 5]. It has been reported that more than 450 million tons of biogas slurry are being used in China each year [6]. Biogas slurry has two main uses for plant production: it is used as a bio fertilizer with high levels of nitrogen (N), phosphorus (P), potassium (K), and other trace elements, and it is as a biological pesticide due to its high levels of amino acids, growth hormones, and antibiotics, all of which promote plant growth [7, 8]. It has been reported that biogas slurry contains abundant nitrogen, which is a readily available nutrient. After fermentation, the content of ammonium ions (NH\textsubscript{4}\textsuperscript{+}) and pH of the biogas slurry increased, while the concentration of carbon (C) from the dry matter decreased, and the C/N ratio also decreased [9, 10]. Furthermore, biogas slurry supplies more plant-readily available N than other fertilizers [11]. The available nitrogen can be directly absorbed by plants, including inorganic nitrate (NO\textsubscript{3}\textsuperscript{-}) and ammonium (NH\textsubscript{4}\textsuperscript{+}) and as simple structured organic partly from the degradation of organic matter.

Camellia oleifera Abel (C. oleifera) is an oil tree species that is native to China with a distribution in 18 provinces/cities and C. oleifera is cultivated in more than 1,000 districts in China. It has been reported that the planting area of C. oleifera in China exceeds 65 million acres [12]. Camellia oil, the product of C. oleifera, is a high quality edible oil that is characterized by abundant unsaturated fatty acids, including oleic acid and linoleic acid [13, 14]. China has a long tradition of cooking with Camellia oil, especially in South China. In recent years, the area planted with C. oleifera is expanding since the demand for oil is increasing [15]. One of the key factors that determine the yield of C. oleifera is fertilization [16]. Traditional cultivation methods mainly depend on chemical fertilization, farm insecticides, and chemical growth hormones, all of which could lead to acidification and hardening of soil, nutrient imbalance, and regression, which ultimately results in production recession [17–19].

Positive effects of biogas slurry and other organic material on plants and crops have been documented [20]. Liquid fermented biogas slurry, from the outlet of the biogas digester, can be readily used and directly applied to crops, vegetables, fodder grass, and many other plants [21–23]. However, specific knowledge about the effects of biogas slurry on the production of C. oleifera and the soil nutrients remains limited. The potential benefits of biogas slurry for C. oleifera and its application at different amounts need to be elucidated. This study investigated the effects of biogas slurry applications on the soil nutrients, the fruit yield, and fruit quality of C. oleifera to assess whether biogas slurry could partly or wholly substitute chemical fertilizers.

Materials and methods

Ethics statement

This study was conducted in a private C. oleifera plantation in Wannian, Jiangxi province in China from 2015 to 2016. The owner of the land and plantation had given permission to conduct the study on this site.

Materials

The investigated area has a typical warm and humid subtropical monsoon climate with an annual mean temperature of 17°C, an annual rainfall level of 1808 mm, and an annual relative humidity of 82%. The annual number of mean frost-free days is 259 d in the experimental area. C. oleifera trees were planted in red clay soil on sunny and hilly land with a gradient of less than 20%. Ganwu strains were used in this study, the plantation was seven years old, the row spacing was 3 by 3 m, and trees had a height of 2–3 m. The biogas slurry was fermented...
from pig farm yard manure, using a farm biogas digester with a 200 m³ capacity for 30 days. The average characteristics of biogas slurry were detected, as: pH = 8.040 ± 0.020, total N = 0.680 ± 0.032 g/kg, total P = 0.086 ± 0.007 g/kg, total K = 3.620 ± 0.041 g/kg, ammonium = 0.522 ± 0.066 g/kg, percent of organic matter = 0.042 ± 0.003%.

Experimental design

The experiment was conducted using a randomized block design with five treatments according to the level of applied biogas slurry: (1) no biogas slurry [group B0]; (2) 10 kg of biogas slurry/plant/year [group B1]; (3) 20 kg of biogas slurry/plant/year [group B2]; (4) 30 kg of biogas slurry/plant/year [group B3]; (5) 40 kg of biogas slurry/plant/year [group B4]. All five treatments did not receive fertilization with chemical fertilizers. The biogas slurry was fertilized three times a year (March, June, and September) with the furrow method into the drip line of trees (Table 1). The biogas slurry was weighed according to the required amount for each treatment, and mixed with the same weight of clean water and applied to each plot. Each treatment was conducted with three blocks with five replicate plants per plot.

Soil collection and physical-chemical analyses

Immediately after fruit harvest, mixed soil samples were collected from five replication plants in each plot. These soil samples were cleared of roots and all other organic debris and subsequently air-dried, ground, and sieved (1 mm) for analysis of soil available nutrition and further sieved (0.149 mm) for organic matter detection. Organic matter was estimated via organic carbon using the conventional conversion: organic matter = 1.724 × organic carbon; organic carbon was determined by the Walkley-Black wet oxidation method; available N was estimated with the Kjeldahl method; available P was extracted with 1 M NH₄F and 0.5 M HCL and estimated via the molybdenum-antimony colorimetric method; available K was extracted with neutral 1M NH₄OAC and was estimated by flame emission spectroscopy [24].

Fruit collection and analysis

Fruits were harvested in October and single tree yield was calculated by immediately weighing all fresh fruit from each tree. Thirty representative fruit samples of each tree were collected. Because *C. oleifera* yield fluctuates each year [25, 26], the production trait indices were calculated by using the average statistics of two years (2015 and 2016). After seeds were dried at 80°C to constant mass, these were weighted and powdered by a high speed disintegrator with high rotation (TW100, Taisite, China). About 1.0000 g of ground sample was weighed as w₀ (g), then transferred to Soxhlet extraction using petroleum ether (60–90°C) at 80°C for 12h. After the solvent was evaporated in vacuum, the residual was dried at 60°C to a constant weight of w₁ (g) in vacuum. The oil weight was calculated according to the formula: oil weight = w₀—w₁. Fatty acids in fresh fruits were measured according to the Chinese Standard (GB-5009, 168–2016 method), by a gas chromatograph (GC-2010 Plus, Shimadzu, Japan) [27–29].

Table 1. Experiment design (Unit: Kg/plant).

| Treatments | March | June | September | Annual total |
|------------|-------|------|-----------|--------------|
| B₀         | 0     | 0    | 0         | 0            |
| B₁         | 4     | 3    | 3         | 10           |
| B₂         | 8     | 6    | 6         | 20           |
| B₃         | 10    | 10   | 10        | 30           |
| B₄         | 14    | 13   | 13        | 40           |

https://doi.org/10.1371/journal.pone.0208289.t001
following fruit characteristics were evaluated as follows: moisture rate of fresh seed = (fresh seed weight—dry seed weight) / fresh seed weight × 100%, fresh seed rate = (fresh seed weight / fresh fruit weight) × 100%, dry seed rate = (dry seed weight / fresh fruit weight) × 100%, oil rate of kernel = (oil weight / kernel weight) × 100%, oil rate of fresh fruit = oil rate of kernel × dry seed rate × 100%.

**Statistical analysis**

The concentrations of organic matter, available N, P, and K between 2015 and 2016 were statistically analyzed by ANOVA using SPSS 19.0. Means were compared by least significant difference (LSD) tests at p < 0.05, and the data in the result represent the average ± STD. The effects of biogas slurry on soil nutrients (organic matter, available N, P, and K) and yield were tested via correlation analysis.

**Results**

**Effects of biogas slurry on organic matter in rhizosphere soil**

Fertilization with biogas slurry significantly increased the organic matter concentration of rhizosphere soils (Fig 1A and S1 Table). During the first experimental year (2015), concentrations of soil organic matter increased with increasing dose of biogas slurry. Compared to the control group B₀, the organic matter concentration of B₁, B₂, B₃, and B₄ groups increased by 32.2% (p<0.05), 55.8% (p<0.01), 70.9% (p<0.01), and 72.6% (p<0.01), respectively. Multiple comparisons showed significant increases of soil organic matter between the fertilized groups and control group; no significant differences were found among treatments B₂, B₃, and B₄. During the second experimental year (2016), the situation was similar to that in 2015. All biogas slurry application rates led to increased organic matter compared the control group. Treatments B₃ and B₄ achieved higher enhancement rates than those in 2015, with increments of 142.28% and 137.56%, respectively.

**Effects of biogas slurry on available nitrogen in rhizosphere soil**

During the experiment, soil available nitrogen decreased from 2015 to 2016 in the control group. Fertilization with biogas slurry increased soil available nitrogen both in 2015 (p = 0.009) and 2016 (p = 0.000) (Fig 1B and S1 Table). When compared to the control group, treatment B₄, (the treatment with the most biogas slurry fertilization), resulted in the highest improvement of available nitrogen in both 2015 (249.07%) and 2016 (499.2%). Low slurry application (B₁, and B₂) led to similar levels of soil available N in the two experimental years; however, at the second highest slurry addition rate (B₃), soil available nitrogen continued to increase in 2016.

**Effects of biogas slurry on available phosphorus in rhizosphere soil**

All four biogas slurry fertilized groups had higher concentrations of available phosphorus in 2016 than in 2015 (Fig 1C and S1 Table). However, the control group had lower available phosphorus concentrations in 2016 than in 2015. In 2015, the four fertilized groups (B₁, B₂, B₃, and B₄) had increments of 151.81%, 139.97%, 119.96%, and 135.82% of available P, respectively, compared to the control group. Multiple comparisons showed no significant differences among the four fertilized groups in 2015. In 2016, compared to the control group, the enhancement of available P was larger with higher slurry addition rates (B₁, B₂, B₃, and B₄) with increments of 161.95%, 188.88%, 210.10%, and 255.05%, respectively.
Effects of biogas slurry on available potassium in rhizosphere soil

Available K decreased from 2015 to 2016 in both the control and the lowest slurry addition (B₁) treatments (Fig 1D and S1 Table). In 2015, available K in soils increased with increasing amount of biogas slurry application, especially for treatments B₃ and B₄ (43.46% and 49.68%, respectively). In 2016, treatments B₃ and B₄ still showed significant enhancements of 117.07% (p < 0.01) and 132.52% (p < 0.01), respectively.

Effects of biogas slurry on fruit yield and main economic traits of C. oleifera

The average of fruit yield and oil yield of C. oleifera in both 2015 and 2016 showed highest enhancement when at least 30 kg biogas slurry per plant each year was used (Table 2 and S2 Table). A growth trend in oil yield was found in response to increasing biogas slurry.
Table 2. Effects of biogas slurry on yield and the main properties of *C. oleifera*.

| Treatments | Yield kg/plant | Moisture rate of fresh seed (%) | Fresh seed Rate (%) | Dry seed Rate (%) | Oil rate of Kernel (%) | Oil rate of fresh fruit (%) | Oil yield kg/plant |
|------------|----------------|-------------------------------|---------------------|-------------------|------------------------|---------------------------|-------------------|
| B₀        | 1.97±0.60ᵃ      | 44.68±2.03ᵃ                   | 54.39±8.17ᵇ        | 33.84±7.98ᵃ       | 45.24±3.50ᵇ           | 10.52±3.14ᵇ              | 0.20±0.02ᵇ       |
| B₁        | 2.20±1.15ᵃ      | 44.36±2.66ᵇ                   | 59.23±14.07ᵇ       | 33.17±9.13ᵃ       | 49.28±3.16ᵇ           | 10.31±3.92ᵇ              | 0.23±0.12ᵇ       |
| B₂        | 2.04±0.91ᵃ      | 40.49±0.11ᵇ                   | 66.82±15.98ᵇ       | 39.77±9.54ᵃ       | 50.17±1.35ᵇ           | 13.35±2.84ᵇ              | 0.28±0.09ᵇ       |
| B₃        | 2.76±0.76ᵃ      | 42.30±1.33ᵇ                   | 85.23±11.40ᵃ       | 45.59±9.02ᵃ       | 48.77±2.89ᵇ           | 14.40±1.74ᵇ              | 0.41±0.18ᵇ       |
| B₄        | 2.29±0.39ᵃ      | 42.83±2.63ᵇ                   | 69.78±14.96ᵇ       | 43.44±7.18ᵃ       | 54.83±2.08ᵃ           | 16.72±2.88ᵇ              | 0.39±0.17ᵇ       |

Note: Different small letters indicate the significant differences among five levels of biogas slurry addition.

https://doi.org/10.1371/journal.pone.0208289.t002

application. Regarding the main economic traits of *C. oleifera*, the fresh seeds from treatments B₀ and B₁ contained the highest moisture ratios, and the lowest oil yield. Compared to the control treatment B₀, the fruit yield of B₂ and B₄ increased by 40.1% and 16.24%, respectively, and the oil yield increased by 105% and 95%, respectively.

**Effects of biogas slurry on fatty acids in *C. oleifera* oil**

Saturated fatty acids mainly constitute of palmitic acid and stearic acid, and accounted for about 10% of the fatty acid content of *C. oleifera* oil in the present study (Table 3 and S1 Table). The effect of biogas slurry application on stearic acid was close to the significance level (p = 0.07), while it did not affect the unsaturated fatty acid content of fruit (Table 3 and S3 Table). Correlations among saturated and unsaturated fatty acids showed that oleic acid was negatively correlated with palmitic and linoleic acids, while linoleic acid was positively correlated with α-linolenic acid (Table 4 and S4 Table).

**Correlations of soil nutrients and fruit economic traits**

The oil rate of fresh fruit was positively correlated with soil nutrients in 2015 (N, P, and K) and 2016 (organic matter; Table 5 and S5 Table). Oil rates of kernels were positively correlated with the concentrations of available P in both 2015 and 2016, and positively correlated with organic matter in 2015.

**Discussion**

*C. oleifera* is a woody tree species that is endemic to China, and an important economic plant. Camellia oil, the product of *C. oleifera*, is known to benefit health and is commonly used as

Table 3. Saturated and unsaturated fatty acids in the fruit of *C. oleifera* fertilized by different amounts of biogas slurry.

| Treatments | Saturated fatty acid % | Unsaturated fatty acid % |
|------------|------------------------|--------------------------|
|            | Palmitic acid (C16:0)  | Stearic acid (C18:0)     | Oleic acid (C18:1) | Linoleic acid (C18:2) | γ-linolenic acid (C18:3) | α-linolenic acid (C18:3) |
| B₀        | 8.713±0.23ᵃ            | 2.127±0.17ᵃ              | 79.743±1.25ᵃ       | 7.416±1.170ᵃ        | 0.005±0.001ᵃ             | 0.277±0.007ᵇ       |
| B₁        | 7.925±0.33ᵇ            | 1.927±0.152ᵇ             | 78.695±0.574ᵇ      | 7.804±0.485ᵇ        | 0.007±0.001ᵇ             | 0.232±0.017ᵇ       |
| B₂        | 7.927±0.42ᵇ            | 2.034±0.057ᵇ             | 79.620±0.984ᵇ      | 7.286±0.284ᵇ        | 0.005±0.002ᵇ             | 0.260±0.013ᵇ       |
| B₃        | 8.009±0.43ᵇ            | 1.820±0.059ᵇ             | 79.674±1.629ᵇ      | 7.695±1.308ᵇ        | 0.004±0.002ᵇ             | 0.301±0.033ᵇ       |
| B₄        | 7.982±0.13ᵇ            | 2.032±0.277ᵇ             | 80.616±0.485ᵇ      | 6.533±0.511ᵇ        | 0.005±0.000ᵇ             | 0.241±0.031ᵇ       |

Note: Different small letters indicate the significant differences among five levels of biogas slurry addition.

https://doi.org/10.1371/journal.pone.0208289.t003
food in China. Because flowers and fruits of *C. oleifera* grow throughout the year, and mature at the same time, fertilizers must be added to increase yields, especially of fruit and oil [30]. It has been reported that P, N, K, Ca, and Mg are the primary soil nutrients that limit the yield of *C. oleifera* [31]. In conventional planting, chemical fertilizers are widely used, which could result in land retirement, nutrient deficits, and the sealing of soil, and thus led to a reduction of yield [32]. To address this problem, this study used biogas slurry as alternative fertilizer, in *C. oleifera* plantation, and investigated the response of nutrients in rhizosphere soil as well as the yields of *C. oleifera*.

Biogas slurry is a secondary product of anaerobic digestion of bio-materials, and plays a central role in the efforts to improve the utilization of animal manure and to reduce the influence of animal excretion on surrounding environments [33, 34]. During manure digestion, about half of the carbon is released as methane and carbon dioxide (biogas), and part of the organic nitrogen is released as ammonium [35]. When it is applied to fields, ammonium can directly be utilized by crops. Furthermore, biogas slurry contains abundant available N, P and K, which are important nutrients for plants. It has been reported that the supply of N from digested slurry exerts a direct influence on the yield during the growing season, while the supply of P and K can be measured in the next year or the next several years [36]. Therefore, this

### Table 4. Correlations among saturated and unsaturated fatty acids.

| Fatty acid          | Correlation (significance: p-value) |
|---------------------|-------------------------------------|
|                      | Palmitic acid (C16:0) | Stearic acid (C18:0) | Oleic acid (C18:1) | Linoleic acid (C18:2) | γ- linolenic acid (C18:3) | α-linolenic acid (C18:3) |
| Palmitic acid (C16:0) | 1                             |                     |                   |                     |                         |                      |
| Stearic acid (C18:0) | -0.359 (0.189)               | 1                   |                   |                     |                         |                      |
| Oleic acid (C18:1)  | -0.628* (0.012)              | 0.273 (0.325)       | 1                 |                     |                         |                      |
| Linoleic acid (C18:2)| 0.441 (0.099)                | -0.271 (0.328)      | -0.924** (0.000) | 1                   |                         |                      |
| γ- linolenic acid (C18:3)| 0.192 (0.493)        | -0.261 (0.347)      | -0.211 (0.451)   | 0.083 (0.769)       | 1                       |                      |
| α-linolenic acid (C18:3)| -0.174 (0.535)            | -0.132 (0.639)      | -0.375 (0.168)   | 0.567* (0.027)      | 0.036 (0.898)           | 1                      |

Note
* indicates p<0.05, ≥0.01
** indicates p<0.01.

https://doi.org/10.1371/journal.pone.0208289.t004

### Table 5. Correlations of oil yield components and soil nutrients.

| Nutrients of soil | Year | Oil rate of fresh fruit | Oil rate of kernel | Oil yield |
|-------------------|------|------------------------|--------------------|-----------|
| Available nitrogen| 2015 | 0.241 (0.387)          | 0.549* (0.034)     | 0.407 (0.132) |
|                   | 2016 | 0.514* (0.050)         | 0.720** (0.002)    | 0.418 (0.121) |
| Available phosphorus| 2015 | 0.472 (0.076)          | 0.628* (0.012)     | 0.636* (0.011) |
|                   | 2016 | 0.623* (0.013)         | 0.681** (0.005)    | 0.719** (0.003) |
| Available potassium| 2015 | 0.454 (0.089)          | 0.648** (0.009)    | 0.252 (0.364)  |
|                   | 2016 | 0.591* (0.020)         | 0.647** (0.009)    | 0.392 (0.148)  |
| Organic matter    | 2015 | 0.681** (0.005)        | 0.619* (0.014)     | 0.644* (0.010) |
|                   | 2016 | 0.372 (0.172)          | 0.565* (0.028)     | 0.385 (0.157)  |

Note
* indicates p<0.05, ≥0.01
** indicates p<0.01.

https://doi.org/10.1371/journal.pone.0208289.t005
study used a two-year experimental period to investigate the effects of biogas slurry on available N, P, and K of soils and the resulting yield of *C. oleifera*. During the two-year observation, fertilization with biogas slurry had positive effects on the increment of available N, P, and K of soils, and also improved the fruit and oil yields of *C. oleifera*. The results of this study indicated biogas slurry as an effective substitute for chemical fertilization in *C. oleifera* production.

Biogas slurry has an abundance of mineral elements and organic matter that are slowly released. These characteristics of biogas slurry may positively affect soil fertility indices, e.g., organic matter, available N, P, and K over many years [37]. A previous study evaluated the utilization ratio of NH$_4$-N in biogas slurry, and reported that more than 90% of the applied NH$_4$-N could be used, which indicated an immediate increase in the amount of soil NH$_4$-N [9]. Friedel *et al.* reported a 37% increase in inorganic N during the incubation of farmyard manure-derived biogas slurry in soil for 60 days [38]. Similarly, this observed a sharp enhancement of available N in soils fertilized with as little as 10 kg of biogas slurry per plant in 2015. It has been speculated that the amounts of N supplied by biogas slurry in this study exceeded the demand of *C. oleifera*, therefore, available N accumulation was observed in 2016. The positive effects of available P and K after biogas slurry application were in accordance with that of available N. Available P is one of the main ecological factors that limits the increase of *C. oleifera* yield. Yuan *et al.* investigated the response of *C. oleifera* yield to low P and reported that *C. oleifera* roots secreted organic acids in response to low soil P, which led to the utilization of soluble phosphates [39]. This study only found a slight but not sharp decrease of soil available P in the control group in 2016. Kashem *et al.* [40] demonstrated that an alkaline environment could promote the availability of P in soils. It is likely that alkaline biogas slurry in turns facilitates the absorption of P in soils. The slow release of nutrients in biogas slurry could contribute to the accumulation of organic matter, available N, P, and K during the second experimental year. The study predicts a larger promotion of nutrients in rhizosphere soil and yield of *C. oleifera* in response to long-term biogas slurry application.

**Conclusions**

In the present study, the effects of biogas slurry on the nutrients in rhizosphere soil and fruit economic traits of *C. oleifera* have been investigated. Fertilization with biogas slurry significantly enhanced the concentration of available N, P, and K in soils, and significantly improved the yield of *C. oleifera*. During the first year, soils had higher concentrations of N, P, and K after application of biogas slurry and the promotion further continued during the second year. The yield of *C. oleifera* oil also increased over both experimental years, and if more biogas slurry was used, the yield was increased. The yield of oil also showed a correlation to the promotion of soil available N, P, and K in rhizosphere soils. Fertilization with 30 kg/plant/year above (i.e., treatments B$_3$ and B$_4$) had the highest fresh fruit yield, fresh seed rate, and dry seed rate, and resulted in a higher oil yield per plant. Therefore, biogas slurry plays an important role in the production increase of *C. oleifera*, and might be an effective substitution for chemical fertilization in *C. oleifera* production.

**Supporting information**

S1 Table. Corresponding raw data of Fig 1.
(XLSX)

S2 Table. Corresponding raw data of Table 2.
(XLSX)
Acknowledgments

The authors have no conflicts on this study and manuscript, and also appreciate the efforts of Dr. Deping Song from the Jiangxi Agricultural University China and Dr. Evan Siemann from the Rice University USA for revising the manuscript.

Author Contributions

Conceptualization: Dongnan Hu.

Data curation: Lu You, Shuqin Yu, Huiyun Liu.

Investigation: Lu You, Chutian Wang, Zengliang Zhou.

Writing – original draft: Lu You, Ling Zhang.

Writing – review & editing: Lu You, Dongnan Hu.

References

1. Zhang C, Su H, Baeyens J, Tan T. Reviewing the anaerobic digestion of food waste for biogas production. Renewable and Sustainable Energy Reviews. 2014; 38:383–392.

2. D’Imporzano G, Pili R, Corno L, Adani F. Arundodon ax L. can substitute traditional energy crops for more efficient, environmentally-friendly production of biogas: A Life Cycle Assessment approach. BIORESOURCE TECHNOLOY. 2018; 267:249–256.

3. Holm-Nielsen JB, Al Seadi T, Oleskowicz-Popiel P. The future of anaerobic digestion and biogas utilization. BIORESOURCE TECHNOLOY. 2009; 100(22):5478–5484.

4. Surendra KC, Takara D, Hashimoto AG, Khanal SK. Biogas as a sustainable energy source for developing countries: Opportunities and challenges. Renewable and Sustainable Energy Reviews. 2014; 31:846–859.

5. Li JS, Duan N, Guo S, Shao L, Lin C, Wang HJ, et al. Renewable resource for agricultural ecosystem in China: Ecological benefit for biogas by-product for planting. ECOL INFORM. 2012; 12(11):101–110.

6. Mao C, Feng YZ, Wang XJ, Ren GX. Review on research achievements of biogas from anaerobic digestion. Renewable and Sustainable Energy Reviews. 2015; 45:540–555.

7. Banik S, Nandi R. Effect of supplementation of rice straw with biogas residual slurry manure on the yield, protein and mineral contents of oyster mushroom. IND CROP PROD. 2004; 20(3):311–319.

8. Insam H, Gómez-Bréndon M, Ascher J. Manure-based biogas fermentation residues–Friend or foe of soil fertility? Soil Biology and Biochemistry. 2015; 84:1–14.

9. Terhoeven-Urselmans T, Scheller E, Raubuch M, Ludwig B, Joergensen RG. CO2 evolution and N mineralization after biogas slurry application in the field and its yield effects on spring barley. APPL SOIL ECOL. 2009; 42(3):297–302.

10. Hansen MN, Henriksen K, Sommer SG. Observations of production and emission of greenhouse gases and ammonia during storage of solids separated from pig slurry: Effects of covering. ATMOS ENVIRON. 2006; 40(22):4172–4181.

11. Möller K, Müller T. Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. ENG LIFE SCI. 2012; 12(3):242–257.

12. Liang HY, Hao BQ, Chen GC, Ye H, Ma JL. Camellia as an Oil seed Crop. HORTSCIENCE. 2017; 52(4):488–497.
13. Ma JL, Ye H, Rui Yk, Chen GC, Zhang NY. Fatty acid composition of Camellia oleifera oil. Journal für Verbraucherschutz und Lebensmittelsicherheit. 2011; 6(1):9–12.

14. Zhu XY, Lin HM, Chen X, Xie J, Wang P. Mechanochemical-Assisted Extraction and Antioxidant Activities of Kaempferol Glycosides from Camellia oleifera Abel. Meal. J AGR FOOD CHEM. 2011; 59 (8):3986–3993.

15. Wang X N, Chen YZ, Wu LQ, Liu RK, Yang XH, Wang R, et al. Oil Content and Fatty Acid Composition of Camellia oleifera Seed. Journal of Central South University of Forestry & Technology. 2008; 28 (3):11–17.

16. Chen YZ, Peng SF, Wang XN, Hu XY, He JH, Wang DB. Study of High Yield Cultivation Technologies of Oil-Tea Camellia (Camellia oleifera)——Formulate Fertilization. Forestry research. 2007; 20(5):650–655.

17. Carvalho FP. Agriculture, pesticides, food security and food safety. ENVIRON SCI POLICY. 2006; 9(7–8):685–692.

18. Watts DB, Torbert HA, Prior SA, Huluka G. Long-term tillage and poultry litter impacts soil carbon and nitrogen mineralization and fertility. SOIL SCI SOC AM J. 2010; 74(4):1239–1247.

19. Matson PA, Parton WJ, Power AG, Swift MJ. Agricultural Intensification and Ecosystem Properties. SCIENCE. 1997; 277(5325):504–509. PMID: 20662149

20. Edmeades DC. The long-term effects of manures and fertilisers on soil productivity and quality: a review. NUTR CYCL AGROECOSYS. 2003; 66(2):165–180.

21. Hernández T, Chocano C, Moreno J, García C. Use of compost as an alternative to conventional inorganic fertilizers in intensive lettuce (Lactuca sativa L.) crops—Effects on soil and plant. Soil and Tillage Research. 2016; 160:14–22.

22. Al Seadi T, Drosg B, Fuchs W, Rutz D, Janssen R. 12—Biogas digestate quality and utilization. In: Wellinger A, Murphy J, Baxter D editors, The Biogas Handbook: Woodhead Publishing; 2013.pp.267–301

23. Bond T, Templeton MR. History and future of domestic biogas plants in the developing world. ENERGY SUSTAIN DEV. 2011; 15(4):347–354.

24. Allen SE. Chemical analysis of ecological materials. Oxford: Blackwell Scientific Publication, 1989.

25. Chen, YH, "PHYSIOCHEMICAL PROPERTIES AND BIOACTIVITIES OF TEA SEED (Camellia oleifera) OIL" (2007). MS. Theses, Clemson University. 2007.

26. Liao T, Yuan DY, Zou F, Gao C, Yang Y, Zhang L, et al. Self-Sterility in Camellia oleifera May Be Due to the Prezygotic Late-Acting Self-Incompatibility. PLOS ONE. 2014; 9(6):e99639. https://doi.org/10.1371/journal.pone.0099639 PMID: 24926879

27. Yuan JJ, Wang CZ, Chen HX, Zhou H, Ye JZ. Prediction of fatty acid composition in Camellia oleifera oil by near infrared transmittance spectroscopy (NITS). FOOD CHEM. 2013; 138(2–3):1657–1662. https://doi.org/10.1016/j.foodchem.2012.11.096 PMID: 23411295

28. Su MH, Shih MC, Lin KH. Chemical composition of seed oils in native Taiwanese Camellia species. FOOD CHEM. 2014; 156(3):369–373.

29. Tan C, Ghazali HM, Kuntom A, Tan C, Ariffin AA. Extraction and physicochemical properties of low free fatty acid crude palm oil. FOOD CHEM. 2009; 113(2):645–650.

30. Vela P, Salinero C, Sainz MJ. Phenological growth stages of Camellia japonica. ANN APPL BIOL. 2013; 162(2):182–190.

31. He G, Zhang J, Hu X, Wu J. Effect of aluminum toxicity and phosphorus deficiency on the growth and photosynthesis of oil tea (Camellia oleifera Abel.) seedlings in acidic red soils. ACTA PHYSIOL PLANT. 2011; 33(4):1285–1292.

32. Fan T, Stewart BA, Yong W, Luo JJ, Zhong GY. Long-term fertilization effects on grain yield, water-use efficiency and soil fertility in the dryland of Loess Plateau in China. Agriculture, Ecosystems & Environment. 2005; 106(4):313–329.

33. Rehl T, Lansche J, Müller J. Life cycle assessment of energy generation from biogas—Attributional vs. consequential approach. Renewable and Sustainable Energy Reviews. 2012; 16(6):3766–3775.

34. Hu Y, Cheng H, Tao S. Environmental and human health challenges of industrial livestock and poultry farming in China and their mitigation. ENVIRON INT. 2017; 107(11):111–130.

35. Maqbool S, Ul Hassan A, JavedAkhtar M, Tahir M. Integrated use of biogas slurry and chemical fertilizer to improve growth and yield of okra. SCIENCE LETTERS. 2014; 2(1):56–59.

36. Liu E, Yan CR, Mei XR, He WQ, B SH, Ding LP, et al. Long-term effect of chemical fertilizer, straw, and manure on soil chemical and biological properties in northwest China. GEODERMA. 2010; 158(3–4):173–180.

37. Zheng X, Fan J, Xu L, Zhou J. Effects of Combined Application of Biogas Slurry and Chemical Fertilizer on Soil Aggregation and C/N Distribution in an Ultisol. PLOS ONE. 2017; 12(1):e170491.
38. Friedel JK. The effect of farming system on labile fractions of organic matter in Calcari-Epileptic Regosols. Journal of Plant Nutrition and Soil Science. 2015; 163(1):41–45.

39. Yuan J, Tan XF, Yuan DY, Zhang XJ, Ye SC, Zhou JQ. Effect of Phosphates on the Growth, Photosynthesis, and P Content of Oil Tea in Acidic Red Soils. J SUSTAIN FOREST. 2013; 32(6):594–604.

40. Kashem MA, Akinremi OO, Racz GJ. Extractable phosphorus in alkaline soils amended with high rates of organic and inorganic phosphorus. CAN J SOIL SCI. 2004; 84(4):459–467.