Black Plastic Film Mulching Increases Soil Nitrous Oxide Emissions in Arid Potato Fields

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Abstract: Black plastic film mulching is a common practice for potato production in the arid area of Northwest China. Many studies have reported the significant positive effect of black plastic film mulch on potato harvest, while the effect of black plastic film mulch treatment on soil nitrous oxide (N\textsubscript{2}O) emissions is still unclear. As a consequence, this study aimed to examine the effect of black plastic film mulch treatment on N\textsubscript{2}O emission from arid upland potato fields. With the static chamber-gas chromatography method, soil N\textsubscript{2}O emissions were measured. The results showed that black plastic film mulch treatment significantly increased cumulative soil N\textsubscript{2}O emissions by 21–26% compared with non-mulched treatment. Cumulative N\textsubscript{2}O emission positively correlated with soil temperature, soil moisture, soil CO\textsubscript{2} concentration, and amoA-AOB abundance. This study indicated that black plastic film mulching, mainly through increasing soil temperature and soil moisture, increasing soil carbon dioxide (CO\textsubscript{2}) concentration, and promoting the abundance of nitrification-related functional gene of amoA-AOB, regulated N\textsubscript{2}O emissions. This study also highlighted that the specific soil environment under black plastic film mulch is conducive to N\textsubscript{2}O emissions and lay the foundation for settling the contradiction between food production and greenhouse gas mitigation in upland soils. The negative effects of black plastic film mulching on the environment should be considered in future applications in food production.

Keywords: nitrous oxide emission; plastic film mulch; amoA-AOB

1. Introduction

Nitrous oxide (N\textsubscript{2}O), with 296 times the global warming potential of carbon dioxide within the 100-year time frame [1], has increased by 122% of the pre-industrial level to 329.9 ± 0.1 ppb [2]. Agricultural N\textsubscript{2}O emission is a tremendous global concern, given that agricultural soils contribute 60% of the global anthropogenic N\textsubscript{2}O emissions [3,4]. Potato is the fourth staple food with a 19.3 Mha harvest area worldwide [5]. Potato fields can potentially contribute to global N\textsubscript{2}O emissions.

Black plastic film mulching, a soil temperature retention soil surface management practice, has been widely used [6,7]. Mulching treatment can significantly enhance potato growth and harvest. One of our previous studies indicated that black plastic film mulch could enhance potato harvest by 6–30% [8], while few studies have paid attention to the effect of mulching on N\textsubscript{2}O emissions in arid and semi-arid potato fields. Soils covered with black plastic film mulch had higher soil temperatures compared to non-mulched soils in potato fields [9]. Mulching has been reported to significantly increase N\textsubscript{2}O emissions in the radish field owing to higher temperatures [10]. An exponential relationship between N\textsubscript{2}O flux and the soil temperature has been observed at 0 to 30 °C, while warming can also inhibit nitrification-induced N\textsubscript{2}O emission when soil temperature is over 30 °C [11]. Additionally, extremely high soil surface temperature (>30 °C) under plastic film mulching has been recorded from 10:00 a.m. to 6:00 p.m. during sprout development and vegetative
growth stages in an arid potato field experiment [12]. Plastic film mulching could also reduce cumulative N$_2$O emissions, which may contribute to the unsuitable soil temperature for N$_2$O emission under mulching [13]. The uncertain effect of black plastic film mulching on N$_2$O emission makes it urgent to investigate how this agronomic practice affects N$_2$O emission in arid potato fields.

Compared to non-mulched treatment, black plastic film mulching could only insignificantly reduce N$_2$O emissions owing to lower soil moisture if there is no irrigation during cultivation [10], while black plastic film mulching is always applied with drip irrigation in arid and semi-arid potato fields. In the black plastic film mulching–drip irrigation system, black plastic film mulch could maintain soil moisture at the 0–100 cm soil layer by reducing soil evaporation [14,15]. Topsoil moisture under black plastic film mulch was generally higher than that under non-mulched soil during the whole growing period [16,17]. On the other hand, nitrification and denitrification are two important pathways for N$_2$O production and were mainly driven by nitrification-related microorganisms (amoA-AOA and amoA-AOB) and denitrification-related microorganisms (nirS and nirK) [18,19]. Soil moisture generally regulates the main microorganisms for N$_2$O production. Nitrification can be an important pathway leading to N$_2$O production when soils were incubated in 60% WFPS, and amoA-AOB would play a key role in N$_2$O production [20]. Di suggested that soil moisture had a major influence on ammonia-oxidizing and denitrifying microbial communities and then regulating N$_2$O emissions [19]. Denitrification-related communities (nirS and nirK) and amoA-AOB are reported to be able to grow under very wet soil conditions (130% field capacity) and produce N$_2$O emissions [19]. Therefore, with a higher soil moisture condition under black plastic film, both nitrification and denitrification-related microorganisms may both play an important role in N$_2$O or denitrification-related microorganisms will dominate N$_2$O emission. While previous studies paid more attention to the abiotic parameters regulating the effect of black plastic film mulch on soil N$_2$O emissions, less attention has been paid to N$_2$O-related microorganisms under plastic film mulch.

We hypothesized that black plastic film mulching could increase N$_2$O emission by increasing soil temperature and moisture, as well as N$_2$O-related microorganisms. We also hypothesized that both nitrification- and denitrification-related microorganisms will increase under mulching treatment leading to a higher N$_2$O emission. This study aimed to (1) explore the effect of black plastic film mulching on soil N$_2$O emission in the arid potato field, and (2) reveal the mechanisms of how black plastic film mulching affects soil N$_2$O emission through regulating soil abiotic and biotic parameters.

2. Materials and Methods

2.1. Field Experimental Design

The field experiments were conducted in 2017 and 2018 at Shiyanghe Experimental Station in Gansu Province, China (37°52′ N, 102°50′ E, 1581 m a.s.l.). The region has a continental temperate climate. The mean annual temperature and precipitation are 8.8 °C and 164 mm, respectively. Daily air temperature and precipitation during the experiment are shown in Figure S1. The soil at the site is a sandy loam soil (9.1% clay, 31.3% silt, and 59.6% sand) with a pH of 8.2, a field capacity of 0.26 cm$^3$/cm$^3$, a bulk density of 1.56 g/cm$^3$ and a total soil porosity of 41%. Other basic soil properties before the experiment are given in Table S1.

The experimental design was a randomized block design with three replicated plots (5 m × 5.6 m) for each treatment. Each plot had seven isosceles trapezoid ridges and each ridge had a length of 5 m, a width of 0.8 m, and a height of 0.3 m. Treatment of soil covered with (MC) or without (CK) black plastic mulch was settled. The black plastic film mulch was high-density and airtight with 0.008 mm in thickness, and covered soils before potato seeds plantation. The plastic film mulch was perforated for the transplantation of potato seeds. All plots were irrigated by an irrigation system as described in our previous studies [8,21]. Briefly, plots were irrigated when soil volumetric moisture content at 20 cm soil depth declined to 70% (v/v) of the field capacity [21]. Soil volumetric moisture
contents were monitored by moisture sensors (introduced in Section 2.2). The amount of water irrigated for one irrigation event was 21 mm. Plots of MC treatment were irrigated 21 and 19 times in 2017 and 2018, respectively. Plots of CK treatment were irrigated 24 and 21 times in 2017 and 2018, respectively. All plots received the same fertilizer according to local management, and the fertilization strategies and other agronomic practices are shown in Table S2.

2.2. Soil Temperature and Moisture Measurement

Soil temperatures at 10 cm soil depth were monitored by thermal sensors (200TS, Irrometer Co., Inc., Riverside, CA, USA), and soil volumetric moisture at 20 cm soil depth was monitored by soil moisture sensors (200SS, Irrometer Co., Inc., Riverside, CA, USA). The data logger (900M, Irrometer Co., Ltd., Riverside, CA, USA) recorded the data every 10 min. The following equation calculated water-filled pore space (WFPS):

$$WFPS = \frac{\text{volumetric moisture content}}{\text{total soil porosity}}$$

where total soil porosity is 41%.

2.3. N2O Flux Sampling

Fluxes of N2O were measured by a static opaque chamber-gas chromatography method (Supplementary Method). Briefly, a stainless-steel frame was inserted 5 cm into the soil of one ridge in each plot and included soil and two crops. An opaque polymethyl methacrylate chamber was placed on the pre-installed stainless-steel frames. Five gas samples were collected to calculate N2O flux around 9 a.m. after chamber closure, at a time interval of 4 min about every 5 days. Gas samples were then kept in pre-vacuumed plastic gasbags (Dalian Pulaite gas packing Co., Ltd., Dalian, China) and analyzed for the N2O concentration using a gas chromatograph (GC-2014 series, Shimadzu (China) Co., Ltd., Beijing, China) equipped with an electron capture detector (ECD). The column (SS-2 m × 4 mm Porapak Q (80/100)) and ECD temperatures were maintained at 60 °C and 300 °C, respectively.

Diurnal N2O flux samples were collected every 4–6 h over three days at early, middle, and later growth stages in both experimental years to identify the effect of MC treatment on N2O flux by alternating soil temperature (Supplementary Method). Flux samples between two irrigation events were also collected every day to identify the relationship between N2O flux and soil moisture (Supplementary Method).

Fluxes were calculated with linear function from the change of gas concentration in the chamber during the sampling period by the following equation [21]:

$$F = H \frac{mp}{R(273 + T)} \frac{\partial C}{\partial t}$$

where $F$ is the N2O flux ($\mu$g m$^{-2}$ min$^{-1}$); $H$ is the height of chamber (m); $m$ is the molecular weight of N2O (g mol$^{-1}$); $p$ is the atmospheric pressure (kPa); $R$ is the value of the universal constant; $T$ is the air temperature in the chamber (°C); $\partial C/\partial t$ is the change of gas concentration in the chamber during the sampling period ($\mu$ mol min$^{-1}$).

Cumulative N2O emissions were calculated by trapezoidal integration [22].

2.4. Soil Gas (CO2 and N2O) Sampling

A soil–air equilibration sampler was installed vertically in the soil to a soil depth of 15–20 cm (Supplementary Method and Figure S2). We extracted 20 mL of gas samples between 10:00 a.m. and 10:30 a.m. after soil N2O flux sampling, using a syringe through a three-way stopcock connecting with the silicon tube. Gas samples were then kept in pre-vacuumed plastic gasbags (Dalian Pulaite gas packing Co., Ltd., Dalian, China) and analyzed for the N2O concentration using a gas chromatograph. The three-way stopcock
of the sampler was closed on non-sampling days to avoid the connection of soil air to atmospheric air.

2.5. DNA Extraction and Quantitative PCR

Soil samples were collected at the harvest time in 2018 and stored at −80 °C for subsequent DNA extraction and quantitative PCR analysis. Soil DNA was extracted using 0.5 g soil according to the manufacturer’s protocol with a Fast DNA Spin Kit for Soil (MP Biomedicals, Eschwege, Germany). Copy numbers of amoA-AOA, amoA-AOB, nirS, and nirK genes were determined by quantitative PCR assays. The primers and thermal cycling conditions are given in Table S3.

2.6. Statistical Analysis

The effects of black plastic film mulching treatment on cumulative N\textsubscript{2}O emission, soil temperature, WFPS, and N\textsubscript{2}O-related gene numbers were analyzed by analysis of variance (ANOVA) for the least significant differences (LSD) at \( p < 0.05 \) level. Differences in cumulative N\textsubscript{2}O emission, soil temperature, WFPS, and N\textsubscript{2}O-related gene numbers between black plastic film mulching treatment and non-mulched treatment were analyzed by ANOVA for LSD at \( p < 0.05 \) level. The above statistical analyses were evaluated by SPSS (IBM SPSS statics version 24.0, SPSS Inc., Chicago, IL, USA). Graph drawing and regression models of soil N\textsubscript{2}O responding to soil abiotic and biotic parameters were performed by Origin and R language.

3. Results

3.1. N\textsubscript{2}O Flux and Soil Temperature

Nitrous oxide fluxes first increased and then decreased for both treatments from 8:00 a.m. to 8:00 a.m. on another day (Figure 1a–f). MC treatment significantly \( (p < 0.05) \) affected daily average N\textsubscript{2}O fluxes on all typical days, and significantly increased daily average N\textsubscript{2}O fluxes by 17–100% (Table S4).

![Figure 1. Diurnal variation of N\textsubscript{2}O emission rates and soil temperatures on three typical days at early (a,d,g,i), middle (b,e,h,k), and later (c,f,j,l) growth stage, and relationship between N\textsubscript{2}O emission rate with soil temperature at early (A), middle (B), and later (C) growth stage. The date of the three growth stages is introduced in Supplementary Methods. MC and CK are abbreviations for treatments with or without mulching, respectively. Vertical bars are the standard error of the mean (n = 3).](image-url)

Diurnal soil temperatures on typical days changed by a single-peak pattern (Figure 1g–l). MC treatment significantly \( (p < 0.05) \) promoted soil temperatures on typical days and significantly increased daily average N\textsubscript{2}O fluxes by 1.8–2.7 °C (Table S4).

There is a quadratic relationship between N\textsubscript{2}O emission rates and soil temperatures at the middle and later growth stages (Figure 1B,C). The N\textsubscript{2}O emission rate reached the greatest at about 25 and 29 °C at the middle and later growth stage, respectively. No significant relationship between N\textsubscript{2}O flux and soil temperature was found at the early growth stage (Figure 1A).

3.2. Soil N\textsubscript{2}O Flux Variation between Two Irrigation Events

Soil N\textsubscript{2}O fluxes changed with a decreasing trend after the irrigation event except fluxes measured on 26–30 July (Figure 2). N\textsubscript{2}O fluxes from 26 to 30 July changed with a flat trend.

![Figure 2. Diurnal variation of N\textsubscript{2}O emission rates and soil temperatures on three typical days at early (a,d,g,j), middle (b,e,h,k), and later (c,f,i,l) growth stage, and relationship between N\textsubscript{2}O emission rate with soil temperature at early (A), middle (B), and later (C) growth stage. The date of the three growth stages is introduced in Supplementary Methods. MC and CK are abbreviations for treatments with or without mulching, respectively. Vertical bars are the standard error of the mean (n = 3).](image-url)
Diurnal soil temperatures on typical days changed by a single-peak pattern (Figure 1g–l). MC treatment significantly ($p < 0.05$) promoted soil temperatures on typical days and significantly increased daily average N$_2$O fluxes by 1.8–2.7 $^\circ$C (Table S4).

There is a quadratic relationship between N$_2$O emission rates and soil temperatures at the middle and later growth stages (Figure 1B,C). The N$_2$O emission rate reached the greatest at about 25 and 29 $^\circ$C at the middle and later growth stage, respectively. No significant relationship between N$_2$O flux and soil temperature was found at the early growth stage (Figure 1A).

### 3.2. Soil N$_2$O Flux Variation between Two Irrigation Events

Soil N$_2$O fluxes changed with a decreasing trend after the irrigation event except fluxes measured on 26–30 July (Figure 2). N$_2$O fluxes from 26 to 30 July changed with a flat trend.

WFPS reached the field capacity after the irrigation event and declined to about 70% (v/v) of the field capacity before the next irrigation event (Figures 2 and S5). Meanwhile, WFPS in MC treatment declined slower than in CK treatment after irrigation (Figure S5).

Soil N$_2$O flux had a positive linear correlation ($p < 0.05$) with WFPS (Figure 2c,f). Fluxes of N$_2$O increased with the increase of WFPS when WFPS changed within a range of 39–62%.

### 3.3. Soil Gas Variation

Soil CO$_2$ showed a firstly increasing and then decreasing pattern with two peaks during the experiments (Figure 3). Topdressing brought about a small CO$_2$ peak in both experimental years. MC treatment had higher soil CO$_2$ than CK treatment. Across two years, average soil CO$_2$ concentrations were $5.3–5.9 \times 10^3$ and $3.5–4.0 \times 10^3$ ppm for MC and CK, respectively.
Soil N$_2$O concentration variation for MC and CK treatments were similar during the experiment, with clear patterns of increasing occurring following the topdressing and decreasing after several days after topdressing (Figure 3). MC treatment had the higher average soil N$_2$O concentrations than CK treatment, being 1.7 (MC) and 1.0 (CK) ppm in 2017 and 3.3 (MC) and 3.1 (CK) ppm in 2018, respectively.

Soil N$_2$O concentrations increased as soil CO$_2$ concentrations increased, and this response was best fitted by an exponential model (Figure 3). Soil CO$_2$ could explain 44–92% of the variance in soil N$_2$O concentrations regarding the correlations between soil C$_2$O and soil N$_2$O (Figure 3).

3.4. Soil N$_2$O-Related Microbial Genes

Black plastic film mulching significantly affected amoA-AOB (Figure 4 and Table 1). The abundance of the amoA-AOB gene was $3.76 \times 10^8$ and $3.12 \times 10^8$ copies per gram of soil for MC and CK treatment, respectively. The abundance of amoA-AOB increased by 21% under MC treatment, compared with the value under CK treatment. No significant difference in the abundance of amoA-AOA, nirS, and nirK genes between MC and CK treatment.

3.5. Seasonal N$_2$O Fluxes

Fluxes of N$_2$O changed with fluctuations, and MC treatment had higher N$_2$O fluxes than CK during the study periods (Figure 5). MC treatment significantly ($p < 0.05$) affected cumulative N$_2$O fluxes (Figure 5 and Table 1). Over two study years, MC treatment increased cumulative N$_2$O emission by 21–26%.

The cumulative N$_2$O flux was positively correlated with soil temperature, soil WFPS, soil CO$_2$ concentrations, soil N$_2$O concentrations, and amoA-AOB gene abundance, while negatively correlated with irrigation times (Figure 6).
Soil N2O concentration variation for MC and CK treatments were similar during the experiment, with clear patterns of increasing occurring following the topdressing and decreasing after several days after topdressing (Figure 3). MC treatment had the higher average soil N2O concentrations than CK treatment, being 1.7 (MC) and 1.0 (CK) ppm in 2017 and 3.3 (MC) and 3.1 (CK) ppm in 2018, respectively.

Soil N2O concentrations increased as soil CO2 concentrations increased, and this response was best fitted by an exponential model (Figure 3). Soil CO2 could explain 44–92% of the variance in soil N2O concentrations regarding the correlations between soil CO2 and soil N2O (Figure 3).

### 3.4. Soil N2O-Related Microbial Genes

Black plastic film mulching significantly affected amoA-AOB (Figure 4 and Table 1). The abundance of the amoA-AOB gene was 3.76 × 10^8 and 3.12 × 10^8 copies per gram of soil for MC and CK treatment, respectively. The abundance of amoA-AOB increased by 21% under MC treatment, compared with the value under CK treatment. No significant difference in the abundance of amoA-AOA, nirS, and nirK genes between MC and CK treatment.

![Figure 4. Soil N2O-related gene abundances at the end of the experiment in 2018. Different letters above each bar indicate a significant difference between black plastic film mulching (MC) and non-mulched (CK) treatment at p < 0.05.](image)

![Table 1. Cumulative N2O emissions, soil temperatures, WFPS, and soil N2O-related gene abundances as affected by black plastic film mulching treatment.](table)

| Year | Parameter | MC          | CK          | ANOVA |
|------|-----------|-------------|-------------|-------|
|      | Cumulative N2O emission (kg hm^{-2}) | 5.56 ± 0.15 a | 4.58 ± 0.25 b | *     |
| 2017 | Soil temperature (°C)         | 20.7 ± 0.5 a  | 18.2 ± 0.3 b | *     |
|      | WFPS (%)                      | 48.4 ± 0.4    | 46.1 ± 0.2  |       |
|      | Cumulative N2O emission (kg hm^{-2}) | 5.77 ± 0.13 a | 4.57 ± 0.28 b | *     |
| 2018 | Soil temperature (°C)         | 21.6 ± 0.0 a  | 19.6 ± 0.3 b | *     |
|      | WFPS (%)                      | 48.4 ± 0.1    | 46.7 ± 0.1  |       |
|      | amoA-AOA (10^7 g^{-1} soil)   | 7.96 ± 0.50   | 7.80 ± 0.85 | ns    |
|      | amoA-AOB (10^8 g^{-1} soil)   | 3.76 ± 0.20 a | 3.12 ± 0.04 b | *     |
|      | nirK (10^5 g^{-1} soil)       | 7.34 ± 0.07   | 7.51 ± 0.12 | ns    |
|      | nirS (10^7 g^{-1} soil)       | 4.91 ± 0.03   | 5.00 ± 0.02 | ns    |

1 Note: MC and CK are abbreviations for treatments with or without mulching, respectively. Different letters near the values indicate a significant difference between MC and CK at p < 0.05. Values are means ± SE (n = 3).

*: difference between treatments was significant (p < 0.05); ns: there was no significant difference.
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2018 Cumulative N$_2$O emission (kg hm$^{-2}$) 5.77 ± 0.13 a 4.57 ± 0.28 b 7.34 ± 0.07 3.76 ± 0.20 a 7.96 ± 0.50 5.77 ± 0.13 a

| Year | Treatment | Cumulative N$_2$O emission (kg hm$^{-2}$) |
|------|-----------|----------------------------------------|
| 2017 | MC        | 7.34 ± 0.07                            |
|      | CK        | 3.76 ± 0.20                            |
| 2018 | MC        | 7.96 ± 0.50                            |
|      | CK        | 5.77 ± 0.13                            |

Figure 5. Daily N$_2$O emission rates and cumulative N$_2$O emissions under the soil with (MC) and without (CK) black plastic film mulching. Different letters above each bar indicate a significant difference between MC and CK at $p < 0.05$. Values of the columns and standard errors are shown in Table 1. Vertical bars are the standard error of the mean ($n = 3$).

Figure 6. Correlations of cumulative N$_2$O emission with soil temperature, WFPS, irrigation times, soil CO$_2$ concentrations, soil N$_2$O concentrations, and abundance of amoA-AOA, amoA-AOB, nirK, and nirS genes (a). Conceptual diagrams showing regulators and controls of black plastic film mulching effect on cumulative N$_2$O emission (b).

4. Discussion

The effect of black plastic film mulching (MC) treatment on N$_2$O emission differs based on previous studies. Studies indicated that MC treatment increased [23], reduced [10,13], or did not affect [24,25] N$_2$O emissions compared with non-mulched treatment. The influence of MC treatment on soil environmental conditions varying with the experimental sites could be the reason for the controversial results. Zhao [10] suggested MC treatment reduced N$_2$O emissions in the hot pepper season owing to lower soil moisture. Reduction of the available mineral N due to the enhanced N uptake of plants would be another reason for the limiting of nitrification and denitrification processes under black plastic film mulching [13]. On the other hand, N$_2$O emissions may not differ between the mulched and non-mulched fields, mainly because film mulching did not affect the soil temperature due to the cooling effect of irrigation [25]. While consistent with Yu [13] and Gao [26], our results showed...
that MC treatment significantly increased N$_2$O emissions, mainly through regulating biotic (amoA-AOB) and abiotic (soil temperature, soil moisture, and soil CO$_2$ concentrations) parameters (Figure 6).

In this study, N$_2$O emissions had a quadratic relationship with soil temperatures during the 24-h measurement (Figure 1B,C), and cumulative N$_2$O emissions also showed a positive relationship with average soil temperatures across two experimental years (Table 1, Figure S3 and Figure 6). Our results indicated that soil temperature was a vital abiotic regulator of soil N$_2$O emission. The failure to find a correlation between N$_2$O flux and soil temperature at the early growth stage (Figure 1A) may be attributed to the lower sensitivity of N$_2$O-related microorganisms to soil temperature when sufficient nutrients in soils at the early growth stage [27]. Zhao [10] indicated that N$_2$O emissions could increase with the increase of soil temperature (from 5 to 32 °C), while we found N$_2$O emissions might decrease when soil temperature was above 25–29 °C at middle and later growth stages (Figure 1B,C). Similar results had been reported that N$_2$O emissions would be inhibited when soil temperature was above 30 °C [11]. Soil N$_2$O emissions could be potentially inhibited by black plastic film mulching for extremely high temperatures. Moreover, from the perspective of cumulative N$_2$O emissions during the whole growing season, the positive effect outweighed the negative one of mulching–induced warming on cumulative N$_2$O emissions. The positive linear correlation between cumulative N$_2$O emission and average soil temperature (Figure 6) could support this inference.

Soil moisture was another primary factor affecting N$_2$O emissions. MC treatment could significantly increase soil moisture, which has been reported by lots of studies [8,21]. We also found soil N$_2$O emissions had a positive correlation with WFPS (from 39% to 62%) (Figure 2), which was consistent with that of Zhao [10]. Zhao indicated that the N$_2$O emission rate was exponentially correlated with WFPS when WFPS varied from 20% to 80% [10]. Results also showed that average WFPS had a positive linear correlation with cumulative N$_2$O emissions, which supports our inference that higher soil moisture under MC would be conducive to N$_2$O production. On the other hand, soil moisture was observed to decrease more rapidly after irrigation under CK treatment (Figure S5), which indicated that soil humidity would retain at a higher level for N$_2$O production for a longer time under MC treatment.

As reported that the denitrification process produced the greatest N$_2$O at 70 to 90% WFPS [28], the nitrification process might domain N$_2$O productions in this study (Figure S4). Ammonia oxidation is the first and rate-limiting step in nitrification, and it is catalyzed by an ammonia monoxygenase (AMO) encoded in gene amoA of AOA and AOB [29]. Although several studies have reported amoA-AOA to play an important role in nitrification [29,30], results in this study showed that MC treatment only significantly enhanced the abundance of amoA-AOB (Table 1 and Figure 4), and amoA-AOB abundances were positively correlated with cumulative N$_2$O emissions (Figure 6). It was AOB, not AOA or other denitrification-related microorganisms, that induced the N$_2$O emission under MC treatment. Previous studies also indicated that amoA-AOB–induced nitrification was regarded as the key pathway for N$_2$O production [31]. Higher amoA-AOB–dependent N$_2$O product stoichiometry than amoA-AOA was likely due to the incomplete NH$_3$OH oxidation and nitrifier denitrification, as previously observed in neutral/alkaline soils [31,32]. Since some amoA-AOB had genes encoding a canonical hydroxylamine dehydrogenase and NO reductase and thus could conduct nitrifier denitrification, they played a more important role in these alkaline mulched soils (Figure 6). Notably, CO$_2$ concentrations under MC treatment were higher than those under CK, which could be attributed to that MC treatment enhancing soil microbes’ activity [33,34]. We found significant exponential correlations between soil N$_2$O and CO$_2$ concentrations (Figure 3), and cumulative N$_2$O emissions were significantly correlated with soil CO$_2$ concentrations (Figure 6). As previous studies mentioned, elevated atmospheric CO$_2$ increased soil nitrification rate and shifted ammonia-oxidizing community abundance and structure [35–37]. Elevated CO$_2$ could both significantly shift amoA-AOB and amoA-AOA communities, and amoA-AOB was more
sensitive to the rising CO$_2$ concentration [35]. We infer that high CO$_2$ concentration under black plastic film mulching might be conducive to the growth of amoA-AOB and promote N$_2$O emissions.

From the above analyses, it can be seen that the MC treatment plays a primary role in regulating N$_2$O emissions. This study showed a negative impact of MC treatment on greenhouse gas mitigation. On the other hand, studies have reported a significant positive effect of MC treatment on potato harvest [8]. Therefore, further research should be devoted to examining the impacts of MC treatment on both potato harvests and N$_2$O emissions.

5. Conclusions

This study used the static chamber-gas chromatography method to evaluate the effect of black plastic film mulch treatment on soil N$_2$O emission in the arid potato field. Our results demonstrated that black plastic film mulch treatment can significantly increase soil cumulative N$_2$O emission. We identified that black plastic film mulching promoted soil N$_2$O emissions mainly by altering abiotic parameters including increasing soil temperatures, soil moisture, and soil CO$_2$ concentrations. We also highlighted that it was amoA-AOB, but not other N$_2$O-related microorganisms that regulated N$_2$O emission under plastic film mulching. Further research should be devoted to examining the impacts of black plastic film mulch treatment on greenhouse gas emissions apart from food production.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijerph192316030/s1, Supplementary Methods; Supplementary Results; Table S1: Soil properties of the top 20 cm soil before experiment; Table S2: Fertilization and agronomic practice in this study. Table S3: Primers and thermal cycling conditions used for quantitative PCR; Table S4: Daily average N$_2$O fluxes and daily average soil temperatures as affected by black plastic film mulching treatment on typical days in 2017 and 2018; Figure S1: Dynamics of air temperature and precipitation during the experiment; Figure S2: Schematic diagram of the soil-air equilibration tube; Figure S3: Daily average soil temperature at 10 cm-depth of soils covered with (MC) or without (CK) black plastic film mulch; Figure S4: Variation of WFPS at 20 cm-depth of soils covered with (MC) or without (CK) black plastic film mulch; Figure S5: WFPS at 20 cm-depth of soils covered with (MC) or without (CK) black plastic film mulch between two irrigation events. References [38–43] are cited in Supplementary Materials.

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