Deforestation for oil palm alters the fundamental balance of the soil N cycle

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Abstract

Expansion of commercial agriculture in equatorial regions has significant implications for regional nitrogen (N) budgets. Here we investigate changes in N availability and turnover in Southeast Asia following the replacement of tropical forest with oil palm plantations along a chronosequence of oil palm maturity (3-months to 15-year-old stands) and secondary to primary forest succession in Sabah, Malaysian Borneo. Ten sites were sampled during March and April 2012 and rates of gross ammonium (NH_4^+) and nitrate (NO_3^-) production (mineralisation and nitrification) and consumption (n = 8), potential denitrification and "anaerobic ammonium oxidation" ("anammox") (n = 12) were determined using ^{15}N isotope additions to soil cores and slurries respectively. Gross mineralisation

rates (0.05 – 3.08 g N m^{-2} d^{-1}) remained unchanged in oil palm relative to forests. However, a significant reduction in gross nitrification (0.04 – 2.31 g N m^{-2} d^{-1}) and an increase in NH_4^+ immobilisation disrupt the pathway to nitrogen gas (N_2) production substantially reducing (by > 90%) rates of denitrification and "anammox" in recently planted oil palm relative to primary forest. Potential nitrous oxide (N_2O) emissions were greater than potential N_2 production and remained unchanged across the chronosequence indicating a potentially increased ratio of N_2O:N_2 emission when soils were first disturbed. These results are an important precursor to studies that could yield improved estimates of regional N turnover and loss in Southeast Asia which will have global implications for N biogeochemical cycling.

1. Introduction

Inputs of reactive N to the terrestrial biosphere have more than doubled over the past century and globally, N-fixing crops, chemical fertilisers and fossil fuel combustion have overtaken biological N fixation and lightning as the principal reactive N inputs (Gruber & Galloway, 2008). This has implications that include enhanced greenhouse gas emissions (Park, et al., 2012), surface-water eutrophication (Bouwman, et al., 2002), soil acidification and changes in biodiversity (Bobbink, et al., 2010; Lu, et al., 2010; Pheonix, et al., 2006). Most global analyses of the N cycle and associated environmental problems draw heavily upon inferences from studies in temperate regions where the majority of research has been undertaken to-date (Pheonix, et al., 2006; Bobbink, et al., 2010). However, the combination of population increase and extensive land-use change in tropical regions since 1950 is changing the N cycle significantly. Particularly significant in this respect are increases in deforestation, fertiliser use, and combustion of fossil fuels with attendant increases in the input and mobility of reactive N that are affecting regional N deposition in the tropics (Hietz, et al., 2011; Sullivan, et al., 2014). Of major concern are increased N_2O and NO emissions, produced largely through microbial nitrification and denitrification. However, estimates of soil N turnover and loss through denitrification and N_2 production remain poorly constrained (Groffman, 2012), particularly in tropical regions of Southeast Asia. Understanding the changes in soil N processes associated with the

conversion of tropical forest to agriculture is therefore critical in seeking to improve models of tropical N cycling in a region of global importance to N biogeochemical cycling.

Recent rates of deforestation in Southeast Asia have been higher than in any other tropical region (Achard, et al., 2002; Miettinen, et al., 2011). The expansion of oil palm (*Elaeis guineensis*) plantations over ~17 million ha of the lowland tropics is an important driver of this deforestation resulting in detrimental changes in above-ground biodiversity (Fitzherbert, et al., 2008), and greater air pollution from increased N and carbon emissions (Hewitt, et al., 2009; Carlson, et al., 2013). Rates of microbial N cycling are also likely to be altered by the physical and chemical changes to soil that accompany oil palm development.

Initially, N is lost through forest clearance, burning and land preparation and N mobility increases post-disturbance as soils with little vegetation cover experience leaching and erosion (Malmer, 1996; Yashiro, et al., 2008; Nykvist & Sim, 2009). In situations where they are able to recover naturally, forests appear able to reduce N-deficits through increased N-retention and reduced nitrification and denitrification (Robertson & Tiedje, 1988; Keller & Reiners, 1994; Davidson, et al., 2007; Templer, et al., 2005). However, as forest succession proceeds, N status and cycling rates increase, and with them, the potential for N loss through N gas production (Davidson, et al., 2007). If forests are converted to agricultural use, N imbalances resulting from *inter alia*, harvesting of crops or additions of inorganic fertilisers, complicate attempts to predict N loss and retention pathways (Silver, et al., 2005; Verchot, et al., 2006; Corre, et al., 2006; Burton, et al., 2007).

Only one study to our knowledge has compared rates of gross N cycling in oil palm plantations with adjacent forests. In Sumatra, Allen et al. (2015) found that converting forests to oil palm plantations decreased gross mineralisation rates in clay Acrisols but not in loam Acrisols. Lower net N transformation rates have also been observed in plantations relative to forest soils (Ishizuka, et al., 2005; Templer, et al., 2005). Net rates are the consequence of both production and consumption processes and, as a result, may be a poor measure of gross N transformation. Therefore, gross rates are a better indicator of total N turnover, particularly in soils where N consumption is high. Moreover,

higher N_2O emissions, and potential denitrification, have been observed from oil palm soils relative to native forests (Hewitt, et al., 2009; Kimura, et al., 2012; Melling, et al., 2007; Yashiro, et al., 2007). However, this increase in N-gas emission is inconsistent with the decline in N cycling which is often observed following forest conversion to agriculture in tropical soils (Silver, et al., 2005; Verchot, et al., 2006; Templer, et al., 2005; Neill, et al., 1999). Our study addresses this inconsistency in oil palm plantations for the first time, by investigating how land-use change impacts soil N cycling in Sabah, Malaysian Borneo. The aim of the paper is to test whether conversion from forest to oil palm alters the rate and balance of N cycling processes. Ultimately, however, changes in microbial processing affect N availability and emissions of N_2O, thus our results provide insight into the N status and sustainability of oil palm agriculture.

In this paper we investigate rates of gross mineralisation, gross nitrification and the potential for N loss through N_2 and N_2O production using a space-for-time substitution across a chronosequence of forest degradation and plantation maturity. Specifically, we examine trends in N cycling across this chronosequence to test the hypothesis that land-use change from forest to plantation agriculture decreases rates of soil N turnover. In undertaking this study we anticipated that as plantations mature, N cycling will follow a trajectory of recovery through plantation maturity comparable to that observed in studies of secondary forest succession (Davidson, et al., 2007; Amazonas, et al., 2011) with increasing N accumulation, turnover and loss with time since disturbance.

2. Materials and methods

2.1 Site description and sampling methods

This study was conducted in the Kinabatangan lowlands of Sabah State in Malaysian Borneo (Fig. 1). The climate is humid tropical with a mean annual (2008 - 2013) temperature of 27.4°C and rainfall typically of 2500 – 3500 mm. The wettest months of the year (December to February) are commonly referred to as the "wet season" but monthly rainfall rarely falls below 100 mm. The Kinabatangan administrative district covers 17,800 km^2 and lowland areas (6,630 km^2) have experienced rapid land-use change during the last 50 years. The predominant vegetation was formerly dipterocarp forest,

although primary forest cover declined by 60-90% during the timber boom between 1975 and 1992 (Vincent & Rozali, 2005). During the same period, secondary forest and oil palm plantations increased commensurately, and oil palm plantations now occupy 2,994 km^2 (~ 45%) of the lowland region where our study sites are located (Latip, et al., 2015).

Soil samples were collected at the end of the wet season in March and April 2012 where monthly rainfall was ~280 mm, compared to an annual total of 3,134 mm. Ten sites, each of 3-5 ha, were sampled within a 1,300 km^2 area representing a chronosequence of forest to oil palm plantation transition. In each plot, 12 subplots of 3 m^2 were selected, within which five cores were randomly extracted for determination of edaphic properties. Minimum distance between subplots was 30 m. Cores were extracted using a 4 cm diameter pipe to 10 cm depth and were homogenised after removal of roots.

Plantations in our study area were established on land that had been degraded by forest clearance and biomass burning. Our chronosequence reflects this by showing the transition from primary, through disturbed forest and oil palm development. We sampled one primary forest (PF) and one early-successional forest (ES) clear cut 16 years prior to sampling (Fig. 1). Two secondary forests at intermediate successional stages (MS1 and MS2) were also sampled (see Supplementary Information for detailed site descriptions). The oil palm plantations comprised stands aged 3 months (3M) and 3, 5, 6, 8, and 15 years (3Y - 15Y). Typically, oil palm has an economic life of 25-30 years after which palms are felled and replanted. Our 3M and 8Y sites were second generation plantations, whilst the remainder were first generation. For the 3M plantation, this transition was significant as 15 months prior to sampling, the old palms were uprooted, chipped and turned back into the soil with substantial physical disturbance. Management and planting practices varied between smallholdings (3Y and 15Y) and commercial plantations (3M, 5Y, 6Y and 8Y): 3Y was a recently converted smallholding whereas the palms in 5Y had been planted on mechanically-raised mounds to counter seedling mortalities associated with the high water table. Fertiliser application varied between smallholdings and commercial plantations: the former received sporadic applications of inorganic N (principally urea, $(NH_4)_2SO_4$ or NH_4NO_3) while the latter received bi-annual treatment. Typical fertiliser additions to

mature oil palms in this region range from 280 – 570 kg N-based fertiliser ha^{-1} $year^{-1}$, with smallholdings falling at the lower end of this range and commercial estates in the higher range. Fertiliser additions also vary with plantation maturity. For example, the newly planted palms in 3M received 70 kg of $(NH_4)_2SO_4$ ha^{-1} $year^{-1}$ whilst adjacent, mature palms within the same plantation received 570 kg of $(NH_4)_2SO_4$ ha^{-1} $year^{-1}$ during 2012-2013. We minimised differences in fertilisation regime by sampling at the end of the wet season when fertiliser-N had not been applied to any of the soils at least three, and up to six, months prior to sampling.

In adopting a chronosequence approach to investigate changes to soil N cycling, we assumed that soil properties were similar prior to conversion. In validating this assumption, we adopted several lines of investigation prior to our study. Firstly, we identified suitable study sites that were of similar elevation and slope and on comparable soil associations from regional soil maps. Whilst all sites were at low elevation (< 100 m a.s.l.) and slope, they differed in their topographical location. Seven of our sites (ES, MS1, MS2, 3M, 3Y, 5Y and 15Y) were located on a riparian formation designated as Tuaran, associated with recent alluvium. However, it was not possible to find an undisturbed riparian forest within our study area due to historic shifting agriculture in the Kinabatangan lowlands. Therefore, our primary forest (PF) site and the remaining two plantations, 6Y and 8Y, were on the terra firma Rumidi Association formed from unstratified inter-bedded mudstone and sandstone of volcanic and sedimentary origin (Acres & Folland, 1975). Secondly, we investigated soil properties at depths that were independent of land use within each location. This confirmed that soils in all study sites were of similar texture (i.e. silt-clay loams), lacked clearly defined horizons, and were visually homogenous to depths > 80 cm (i.e. being poorly drained, all soils were of a similar soil colour and had gleyic properties). Soils in the region are mostly classified as Ferric, Orthic and Gleyic Acrisols and Luvisols. Accordingly, we examined historical data for forested soils in this region, which confirmed that carbon, nitrogen, pH and soil texture of our reference soils (Table 1) were statistically indistinguishable from those reported for similar soils under forested land use prior to land use change (Acres & Folland, 1975). Bulk density and moisture were determined by measuring the wet and dry weights of a known soil volume. Water-filled pore space was determined from volumetric water

content and soil porosity assuming a particle density of 2.56 g cm^{-3}. Soil organic matter (SOM) content was determined by loss on ignition, and pH was measured in a slurry using a soil:water ratio of 1:2. Soil texture was measured by laser diffraction after removal of organics (Sperazza, et al., 2004; Mikutta, et al., 2005). Total N content of soils was determined by dry combustion on an elemental analyser (Vario PYRO cube, Elementar, Hanau, Germany) in Paris at BioEMCo's (Continental Ecosystems Biogeochemistry and Ecology) stable isotope facility in the University Pierre and Marie Curie.

2.2 Gross mineralisation and gross nitrification

Gross N transformation rates were measured by isotope pool dilution following ^{15}N addition to intact cores (Davidson et al., 1991). At each site, eight of the twelve subplots were randomly selected for determination of gross N transformation rates and four additional cores extracted per plot: Two for addition of $^{15}(NH_4)_2SO_4$ solution (98% ^{15}N); and two for addition of $K^{15}NO_3$ (98% ^{15}N). Each core received six 1 ml injections of a solution containing 30 μg N ml^{-1}, thereby enriching the soil by ~ 2 μg N g^{-1} (See Supplementary Information). After injection and incubation, cores were homogenised, roots removed by hand, and a subsample extracted with 2M KCl in a 5:1 solution to soil ratio 15 minutes (T_0) and 24 hours (T_1) after ^{15}N addition. Recovery rates are reported in the Supplementary Information. Additional cores, extracted adjacent to, and at the same time, as the T_0 cores, were used to correct for incomplete recovery of ^{15}N immediately after addition (Davidson, et al., 1991). Extraction was done using pre-leached Whatman No.1 filters, and filtrates were immediately frozen until transportation back to the UK for analysis. Analysis of NH_4^+ and NO_3^- was conducted by colourimetric analysis using the salicylate method and the cadmium reduction method respectively (Mulvaney, 1996).

Samples were prepared for ^{15}N analysis by ammonia diffusion and corrected using diffusion blanks (Brooks, et al., 1989; Stark & Hart, 1996). One site, MS2, was excluded from the results for gross nitrification and NO_3^- consumption as N diffused onto the filter discs was below the concentration necessary to measure ^{15}N atom% reliably by isotope ratio mass spectrometry (IRMS). Following this,

samples with low N concentrations were amended with a ^{14}N carrier solution before diffusion to yield a sufficient signal to permit IRMS analysis (Davidson, et al., 1991). Isotope ratios were determined by IRMS on a continuous-flow Isoprime™ IRMS connected to an Elementar PYRO cube© in the University of Birmingham stable isotope facility. Gross mineralisation and gross nitrification as well as NH_4^+ and NO_3^- consumption rates were calculated according to Kirkham and Bartholomew (1954). NH_4^+ immobilization was estimated by subtracting gross nitrification from gross NH_4^+ consumption. Rates of NH_4^+ immobilisation calculated in this way have been shown to be a reasonable approximation of measured microbial immobilisation (Burger & Jackson, 2003).

2.3 Potential N_2 and N_2O production

Potential rates of N_2 production were measured in anoxic soil slurries using ^{15}N tracer techniques without extraneous carbon (Thamdrup & Dalsgaard, 2002; Trimmer, et al., 2003). Duplicate vials were prepared for each sample to allow destructive sampling at 0, 0.5, 1, 1.5 and 2 hours. Approximately 1g of field-moist soil was placed in a 3 ml gas-tight vial (Exetainer, Labco) filled to the top with deionised water and incubated at ~28°C for 24-36 hours. This pre-incubation was to ensure depletion of any ambient $^{14}NO_3^-$ and $^{14}NO_2^-$ before ^{15}N addition (Thamdrup & Dalsgaard, 2002; Trimmer et al., 2003). An injection of 125 µl of 9.8 mM $K^{15}NO_3$ (98% ^{15}N) through the rubber septa of the vials enriched the slurries by 250 µM $^{15}NO_3^-$, which was marginally higher than the mean ambient slurry $^{14}NO_3^-$ concentration prior to pre-incubation of 212 µM $^{15}NO_3^-$ across all sites. Vials were shaken and incubated for 0.5-2 hours before microbial activity was stopped by injection of 100 µl 50% w/v $ZnCl_2$.

Production of ^{15}N-N_2 gas was measured by continuous flow IRMS (Thermo-Finnegan, Delta Matt Plus) following creation of a 1 ml helium headspace above the slurry at Queen Mary University, London (Trimmer & Nicholls, 2009; Lansdown, et al., 2012). The increase in ^{29}N and ^{30}N abundance was determined by reference to control vials that received no ^{15}N in which microbial activity was stopped at the beginning of the experiment. Pre-incubation of the vials will have removed the ambient nitrate, therefore as the added NO_3^- was 98% ^{15}N, the predicted proportion of $^{29}N_2$ from denitrification

should be < 2% (i.e. $p^{29}N = 0.02 + (2*0.98)$). However, many samples showed $^{29}N_2$ in concentrations substantially above the predicted amount indicating that a process other than denitrification was contributing to ^{29}N production. This could have been due to anammox (i.e. $^{14}NH_4^+ + {}^{15}NO_2^- \rightarrow {}^{29}N_2 + 2H_2O$), however, we did not specifically screen for the anammox reaction by the addition of $^{15}NH_4^+$ both with and without $^{14}NO_3^-$. As an alternative process may have contributed to $^{29}N_2$ production, we attribute this $^{29}N_2$ production to an anammox-like reaction. Production of ^{15}N-N_2 was calculated from Thamdrup & Dalsgaard (2002) by:

— — (1)

where p^xN_2 is the excess $^{29}N_2$ or $^{30}N_2$ in the headspace of the Exetainer; / is the ratio of heavy isotope to total N_2 signal for either sample (*s*) or reference (*r*) and α is a calibration factor determined by reference to atmospheric air. The Bunsen coefficient for N_2 was used to account for gas solubility in liquid.

Equation 1 was then used to calculate the total amount of N_2 produced via denitrification and "anammox" through:

(2)

and

(3)

where is the fraction of ^{15}N in NO_3^- (i.e. 98%).

N_2O production was measured from duplicate vials containing slurries that were prepared identically to those used to determine rates of denitrification and "anammox". A 50 µl subsample of the headspace was injected into a gas chromatograph (GC Agilent Technologies UK Limited, Cheshire) equipped with 63Ni electron capture detector using a 5% methane:argon carrier gas and calibrated against known standards. N_2O production was calculated as the increase in concentration versus time using the Bunsen coefficient to account for gas solubility in the liquid phase.

2.4 Statistical analysis

We tested sampling points within each location for spatial independence using data for soil edaphic properties and denitrification and anammox process rates. Tests were performed via ordinary kriging and modelled in ArcMap©, ver.10.0 using weighted least-squares analysis (data not shown). Spatial independence for process rates was found at distances > 3 m in forested sites and > 5 m in oil palm sites. We also found that for most edaphic variables (pH excluded), spatial independence was achieved at distances > 5.5 m in plantation sites and substantially less than this for forested sites. pH was independent at distances > 13.5 m and > 15 m in plantation and forested sites respectively. Therefore, our sampling points randomly spaced at distances > 30 m apart were spatially independent and were considered replicates for statistical testing. Data were checked for normality using the Shapiro–Wilk test and variables displaying non-normality were log transformed before analysis. N cycling processes are reported as an aerial rate (m^{-2} to 10 cm depth) using the mean and standard error as measures of central tendency and distribution after adjusting for bulk density. When converting to an aerial rate, the bulk density for the site was used rather than the reference bulk density of the PF. Analysis was performed using one-way ANOVA with sites representing treatments. Multiple comparisons of means were then completed using Tukey's post-hoc test. However, correlation analysis was performed using the Spearman's rank test on process rates prior to adjusting for bulk density. All significance levels are reported as $p < 0.05$ and analysis was performed in R v.2.15.3 (R Development Core Team 2013).

3. Results

3.1 Site and edaphic factors

Although all soils were classed as silt-clay loams, there were some differences in the sand ($p < 0.001$) and clay content between sites ($p < 0.001$). Most notable were the low sand content of 6Y and 15Y (Table 1). Despite differences across sites, clay content was within 10%: ranging from 21- 31%. Bulk density in 6Y was significantly higher than the in the undisturbed soils of the PF, and the recently converted 3Y. Unlike many of the commercial plantations that employ heavy machinery to remove

vegetation and prepare sites, 3Y had been largely cleared by hand with limited soil disturbance. Residual forest vegetation (i.e. tree stumps), and a vigorous regrowth of herbaceous vegetation was also observed at this site. Soil pH ranged from a low of 4.34 ± 0.03 in 15Y to a significantly high 5.91 ± 0.1 in 5Y.

Soils in forested sites had similar properties with the exception of soil organic matter and N content. Specifically, soil texture, bulk density, pH, C:N ratio and inorganic N (NO_3^- and NH_4^+) content did not differ regardless of forest successional stage. However, soil organic matter declined with increasing forest disturbance and was significantly higher in the primary forest than more recently disturbed successional forests, MS2 (p = 0.01) and ES (p = 0.002). We found no evidence of organic matter loss or gain through plantation age, but the organic content of soils in 5Y was similar to the primary forest. The high organic content and pH of site 5Y may be a result of drainage, organic matter returns (i.e. straw and empty fruit bunches) and, possibly, liming at this heavily-managed site. Organic matter was lowest in 3Y. The C:N ratio was characteristically low for tropical soils and was statistically lower in 6Y plantation compared to the two most mature plantations (8Y and 15Y), one of the mid-successional (MS1), and the ES forest.

Total organic N (%) correlated with soil organic matter (r = 0.664; p < 0.001) and was highest in the primary forest (Table 1) and lowest in 3M. For 3M, on its second rotation as an oil palm plantation, total N was statistically lower than in all other sites. In the remaining plantations aged 3 - 15 years, soil N ranged from 0.18% – 0.32%, which was similar to that observed in successional forests (0.17% – 0.30%). In forested sites, there was a partial trend of decreasing soil N with increasing forest disturbance (i.e. MS2 > MS1 > PF), and the N content of the two most disturbed forests, ES and MS1, were significantly lower than that of the primary forest.

3.2 Gross N transformation rates

In-situ measurements of NH_4^+ and NO_3^- production and consumption, through isotope pool dilution, provided indices of gross N mineralisation and gross nitrification (Fig. 1). Gross mineralisation rates did not differ significantly across the chronosequence (p = 0.597) although NH_4^+ production tended to

be higher in plantations (mean: 1.5 ± 0.15 g N m^{-2} d^{-1}) than forests (mean: 1.0 ± 0.15 g N m^{-2} d^{-1}). There was also a tendency for NH_4^+ production to decline during the initial stages of plantation development. Specifically, rates in the recently re-planted 3M plantation were approximately half that of the primary forest and one third of that observed in palm stands over 5-years old. Gross mineralisation appeared to recover, albeit partially, in 3Y before stabilising in plantations aged 5 – 15 years. Gross NH_4^+ consumption (See Supplementary Table S2) was similar to, and strongly correlated with, gross mineralisation (r = 0.92; p < 0.001). In general, NH_4^+ was consumed at a lower rate than it was produced yielding positive net mineralisation. Gross NH_4^+ production was correlated with water-filled pore space (r = 0.378; p = 0.003) and soil organic matter (r = 0.320; p = 0.012). High rates of gross mineralisation were accompanied by ammonium as the dominant (5 – 10 times greater than nitrate) form of inorganic N in all soils. Extractable ammonium tended to be higher in the most recently disturbed sites; ES and 3Y (Table 1).

Gross nitrification ranged from 0.34 ± 0.10 g N m^{-2} d^{-1} in the 8Y to 1.39 ± 0.21 in the PF (Fig. 1). In general, rates of gross nitrification in forests were approximately double that of plantations, although the only significant difference was between 8Y and MS1 (p = 0.007). NO_3^- consumption was positively associated with NO_3^- production (r = 0.965; p < 0.001), however consumption exceeded production at all sites resulting in negative net nitrification. Concentrations of extractable NO_3^- were consistent with high nitrate consumption, remaining low across sites, and ranging from 0.19 ± 0.02 g N m^{-2} in MS1 to 0.43 ± 0.20 g N m^{-2} in 15Y. However, nitrate concentrations were generally higher in the plantations relative to the forests (Table 1), and were statistically higher in the 3Y, 6Y and 15Y plantations relative to MS1. Extractable nitrate was not correlated with gross NO_3^- production but was positively correlated with gross mineralisation (r = 0.389; p = 0.002) and total N (r = 0.459; p = 0.001).

In forested sites, gross nitrification exceeded NH_4^+ consumption resulting in negative NH_4^+ immobilisation (Fig. 2). In oil palm sites, the opposite was true as NH_4^+ consumption in excess of gross nitrification resulted in positive immobilisation of ammonium at the majority of sites. Interestingly, the exception to this was the first-generation 3Y smallholding that had most recently

undergone conversion, retained evidence of residual forest vegetation, and had an established herbaceous regrowth. As the calculation of NH_4^+ immobilisation resulted in large standard errors, the majority of sites did not differ statistically. However, the high NH_4^+ immobilisation rate in 6Y and 8Y was significantly greater than the strongly negative rate of the mid-successional forest, MS1 ($p < 0.001$). NH_4^+ immobilisation was positively correlated with water-filled pore space ($r = 0.505$; $p = 0.001$), but did not correlate with any other edaphic property.

3.3 Potential N_2 production rates

Denitrification was responsible for 45-91% of total N_2 production potential (i.e. $^{29}N_2 + {}^{30}N_2$ production) and, for most sites, was the principal N_2 formation mechanism (Fig. 2). Differences between sites were highly significant ($F(9,48) = 27.9$; $p < 0.001$) and denitrification in PF was higher than all other sites. There was also a clear trend of declining denitrification through forest disturbance with the lowest rates observed in 3Y.

Denitrification increased as plantations matured and was statistically higher in 15Y than ES and palm stands aged 3 months to 5 years. Despite the increase in denitrification with stand maturity, rates were still only 43% of the PF reference rate 15 years after plantation establishment. We assumed that recovery of denitrification function is linear through plantation age ([$Denitrification_{rate}$ = 0.0286*plantation age (years) – 0.0006; $r^2 = 0.94$]; See Supplementary Fig. S2) and extrapolated mean rates through the economic life of the plantation. Our model indicates that N_2 production attributed to denitrification will be 0.71 ± 0.07 (i.e.~ 75%) of that of the PF by the time palms are felled, chipped and replanted at 25 years. Although a similar amount of time had elapsed since disturbance in ES and the 15Y, denitrification potential in 15Y was significantly higher than in ES ($p < 0.001$). Denitrification rates were positively correlated with silt content ($r = 0.306$; $p < 0.001$), water-filled pore space ($r = 0.238$, $p = 0.01$), and nitrate ($r = 0.210$; $p < 0.025$). Rates were also negatively correlated with sand content ($r = -0.268$; $p < 0.004$).

Production of $^{29}N_2$ was above that which could be reasonably attributed to denitrification indicating that an alternative process (e.g. anammox or codenitrification) was contributing to N_2 production (Thamdrup & Dalsgaard, 2002; Trimmer, et al., 2003). We attribute this additional N_2 production to an "anammox-like" reaction. Production via this pathway was generally an order of magnitude less than for denitrification. However, unlike denitrification, there was no trend of decline and recovery following disturbance (Fig. 2). The highest rates were observed in the PF which significantly exceeded the rate observed in ES ($p < 0.001$) and in 15Y ($p = 0.045$). Potential activity in 8Y and 5Y was similar to the PF but greater than in 15Y and ES. Production of N_2 via this anammox-like process was significantly correlated with denitrification ($r = 0.564$; $p < 0.001$) and silt content ($r = 0.317$; $p < 0.001$) and negatively correlated with sand content ($r = -0.292$; $p = 0.002$).

3.4 Potential N_2O production

Potential N_2O production in the PF was four times greater than N_2 production (Fig. 2) and remained unchanged across the chronosequence regardless of land use or successional stage. However, the maintenance of N_2O production potential contrasts with that of N_2 production (as the sum of both denitrification and "anammox") potentially resulting in a much greater N_2O:N_2 emission ratio in recently disturbed soils (Fig. 3).

Maximum N_2O:N_2 production ratios were observed for the 3M plantation (N_2O:N_2 = 85 ± 20). As plantations matured, ratios of N_2O:N_2 production declined to pre-disturbance levels (i.e. N_2O:$N_2 \approx 4$ in 15Y), however, rates of potential N-gas emission were lower in 15Y relative to the PF. N_2O data were not available for plantation 6Y.

4. Discussion

4.1 Deforestation and oil palm establishment alters key components of soil N cycling

We observed a fundamental alteration to the balance of key N process rates following deforestation and the establishment of oil palm plantations. In the PF, rates of gross mineralisation were commensurate with rates of gross nitrification and N_2 production signifying a relative equilibrium

between soil internal N turnover and loss. However, disturbance perturbs this balance by decreasing gross nitrification and increasing NH_4^+ immobilisation disrupting the pathway to N cycle closure via inert N_2 production. Conversely N_2O production remains unchanged across the chronosequence potentially resulting in greater N_2O:N_2 emissions in the most disturbed soils.

4.2 Reduced gross nitrification and increased NH_4^+ immobilisation disrupt N_2 production

Only a few studies to date have examined gross N transformations in tropical lowland soils and most of these have been conducted in South America. To our knowledge, only Allen et al. (2015) have measured gross N transformation rates as forests are converted to oil palm. In Jambi Province, Sumatra, the authors found post-conversion reductions in NH_4^+ turnover were apparent in clay Acrisols that had higher initial fertility, but not in loam Acrisols, which were inherently less fertile (Allen et al. 2015). We did not observe any correlation of N cycling rates with soil clay content, however, the observations in Allen et al. (2015), together with those of others (Silver et al. 2000; Sotta et al. 2008), highlight the potential importance soil texture and clay content in soil fertility and N turnover. Gross mineralisation and gross nitrification rates have also been measured in other perennial tropical crops (Garcia-Montiel & Binkley, 1998; Silver, et al., 2005; Corre, et al., 2006; Burton, et al., 2007). In comparison with these studies, gross mineralisation rates in our forest soils were higher than the loam Acrisols and similar to the clay Acrisols observed by Allen et al. (2015). Gross mineralisation rates in our plantations were similar to those of *Albizia* and *Eucalyptus* plantations in Hawaii (Garcia-Montiel & Binkley, 1998). Gross nitrification rates, although high (0.64 – 18 mg N kg^{-1} d^{-1}), are within the range of 0.01 – 24 mg N kg^{-1} d^{-1} reported for tropical soils generally (Neill, et al., 1999; Kiese, et al., 2002; Burton, et al., 2007).

Converting forests to commercial plantations has been associated with an increase in gross mineralisation in sub-tropical Australian hoop pine (Burton, et al., 2007), and a decrease in Costa Rican Spanish elm (Silver, et al., 2005). Statistically, we found no difference in gross NH_4^+ production or consumption after our forests had been converted to oil palm. There was a non-significant decline in gross mineralisation rates immediately following conversion which recovered 5

years after planting indicating that gross mineralisation function is only minimally impacted by land-use change. However, rates of gross nitrification (and NH_4^+ immobilisation) were affected.

Soil nitrification is often assumed to be strongly influenced by land-use change as nitrifiers (specifically, autotrophic nitrifiers) represent a specialised functional group which is sensitive to environmental parameters such as low O_2 availability and low pH (Carney, et al., 2004). Several studies note declining nitrification following conversion of tropical forests to agriculture (Neill, et al., 1999; Silver, et al., 2005; Verchot, et al., 2006; Burton, et al., 2007). The magnitude of decline in our agricultural soils (~50%) relative to forests is similar to that reported for the conversion of forest to hoop pine plantations in Australia (Burton, et al., 2007), although the decline was statistically significant between only one forest and one plantation. However, substantial NO_3^- production across all sites indicates that nitrification is an important process, particularly for our forested sites where gross nitrification rates exceeded those of gross mineralisation. Rates of gross nitrification in excess of gross mineralisation are unusual but not without precedent (Accoe, et al., 2005; Burton, et al., 2007). Burton et al (2007) attributed rates of gross nitrification > gross mineralisation to heterotrophic nitrification which may be important in some acidic forest soils where the environmental conditions, such as low pH, inhibit autotrophic nitrification (Zhang, et al., 2011; Zhu, et al., 2013).

The contrast between gross NH_4^+ consumption and gross nitrification led to substantially different estimates of NH_4^+ immobilisation in our forests and plantations. Recent studies in Sabah suggest that soil microbial community dynamics are altered following conversion of forests to oil palm, but that secondary forests retain similar microbial communities to primary forests (Lee-Cruz, et al., 2013; Kerfahi, et al., 2013). While we did not characterise the microbial community directly, potential denitrification is an indicator of microbial denitrifying function. In our soils, this function was drastically reduced (i.e. by > 90%) in disturbed forests and plantations relative to the PF. Thus, it is possible that patterns in gross nitrification and NH_4^+ consumption reflect a change in the microbial community. For example, fungi appear particularly vulnerable to land-use change: Kerfahi et al. (2013) found that fungi associated with decomposition of recalcitrant substrates, such as large woody debris in forested sites, were less abundant following conversion to oil palm. As fungi have a wider

C:N ratio than bacteria (e.g. 10 versus 4), they assimilate N more efficiently than their bacterial counterparts. Where bacteria increase at the expense of fungal populations, this potentially results in increased ammonium consumption. Furthermore, loss of ecosystem functionality has been observed through deteriorating root community traits following the conversion of forests to oil palm by Sahner et al, (2015). Therefore if land-use change preferentially affects one microbial functional group, for example, fungi or heterotrophic nitrifiers compared to decomposers, this may explain the decline in gross nitrification and consequently the switch to positive NH_4^+ immobilisation in our plantations. Site 3Y is the most recently converted oil palm plantation (3M being a second generation site) that we sampled, and the microbial community of 3Y is likely to be most similar to that of forested sites, which could explain the persistence of negative NH_4^+ immobilisation in this one plantation.

4.3 Declining N_2 production increases the potential for emissions of N_2O

High gross nitrification rates suggest that NH_4^+ released by mineralisation is likely to be rapidly nitrified in these soils. For the PF, rates of denitrification were similar to rates of gross nitrification, and likewise between rates of gross nitrification and gross mineralisation. This balance in the N cycle suggests that denitrification is the likely fate for nitrate in undisturbed forest soils. Conversely, in disturbed soils, decreased gross nitrification coupled to increased NH_4^+ immobilisation creates an imbalance between these key N cycling components resulting in a significant (57-98%) reduction in N_2 production. By disrupting completion of the N cycle through production of inert N_2, this sink for NO_3^- is substantially diminished following land-use change. Furthermore, high potential nitrification and high N_2O production increased the likelihood of emissions of this greenhouse gas succeeding a large influx of N such as that following fertilisation of oil palm plantations. Our results indicate that the higher N-gas emissions observed in plantations relative to forests by others (Hewitt, et al., 2009; Kimura, et al., 2012; Melling, et al., 2007; Yashiro, et al., 2007), is unlikely to result solely from increased denitrification capacity in plantation soils. Whilst N_2 production declined, potential N_2O emission rates were unaffected by soil disturbance and land-use change suggesting that the initial stages of denitrification (i.e. reduction of NO_3^- to NO_2^-, NO and N_2O) are relatively resistant to disturbance. However, the decline in N_2, but not N_2O, production capacity may have implications for

the proportion of N_2O emitted during denitrification. Whilst our rates of N_2O emission are potential rates in soils incubated for a short (0 – 2 hour) period, and therefore may not reflect in-situ rates of N_2O production, they nevertheless demonstrate an increased risk of N_2O:N_2 production during initial soil disturbance. This potential for increased N_2O relative to N_2 emission is also likely to be exacerbated following fertilisation of oil palms.

4.4 Does N cycling "recover" through plantation maturity?

We expected to observe a recovery of N status and an increase in N cycling as plantations matured, however plantations showed little temporal direction to N status with the exception of potential denitrification. Furthermore, rates in 15Y were 43% of the PF rate. Even if recovery continues with stand maturity, there is insufficient time for denitrification function to return to primary forest rates before replanting occurs at ~25 years. In addition, recovery of denitrification in successional forests was slower than in plantations. In contrast to 15Y, ES was cleared 16 years before sampling, yet denitrification rates were only 16% of 15Y. Slow recovery under secondary succession might result from the net increase in ecosystem productivity within regenerating forests that creates demand for N and reduces rates of N loss (Brearley, 2011; Templer, et al., 2005). By contrast, plantations may be less limited by N due to fertilisation, as even relatively modest applications of inorganic N are likely to be greater than natural inputs through biological N fixation.

Rates of N_2 production via our anammox-like process were comparable, or slightly higher, than those reported for the Luquillo tropical forest (Yang, et al., 2012), but were an order of magnitude less than denitrification. The unsystematic decline, or recovery, in activity through disturbed forests and plantations suggests that this process has lower resistance and resilience than denitrifiers to the stresses imposed by land-use change. The disturbance of primary forests and establishment of plantations may therefore have a greater detrimental effect on N_2 production via this process than denitrification but the effect on overall production rates of N_2 is less.

In conclusion, our results show that the relative balance of N turnover and loss in the primary forest is disrupted following land-use change. While gross NH_4^+ production was similar in both forests and

plantations, reduced gross nitrification and increased ammonium immobilisation affected the soil's ability to close the N cycle through N_2 production post-disturbance. Furthermore, using a space-for-time substitution indicates this balance is unlikely to be restored during the economic life of the plantation. The potential for N_2O production was greater than N_2 and remained unaffected by disturbance. This has important implications for potential N_2O:N_2 emission ratios, particularly during the early years of plantation establishment and following applications of fertiliser to oil palm. The switch from negative NH_4^+ immobilisation in forests to positive immobilisation in plantations may also result from changes to microbial population dynamics and warrants further investigation.

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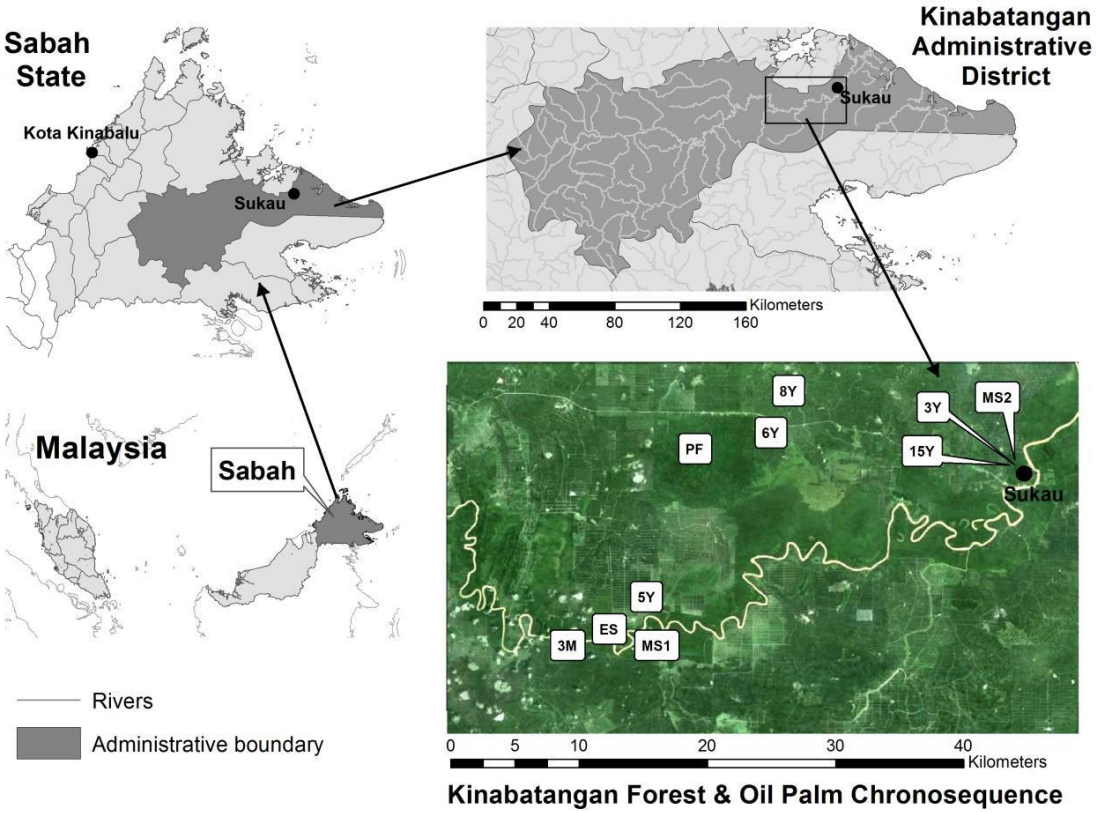

Figure 1: Location of the study site within Sabah State and Kinabatangan District and the location of the sampling points on the landscape. Forested sites (in ascending order of succession) are early successional (ES), mid-successional 1 and 2 (MS1 and MS2) and primary forest (PF). Oil palm sites are stands aged 3M (months), 3Y, 5Y, 6Y, 8Y and 15Y (years).

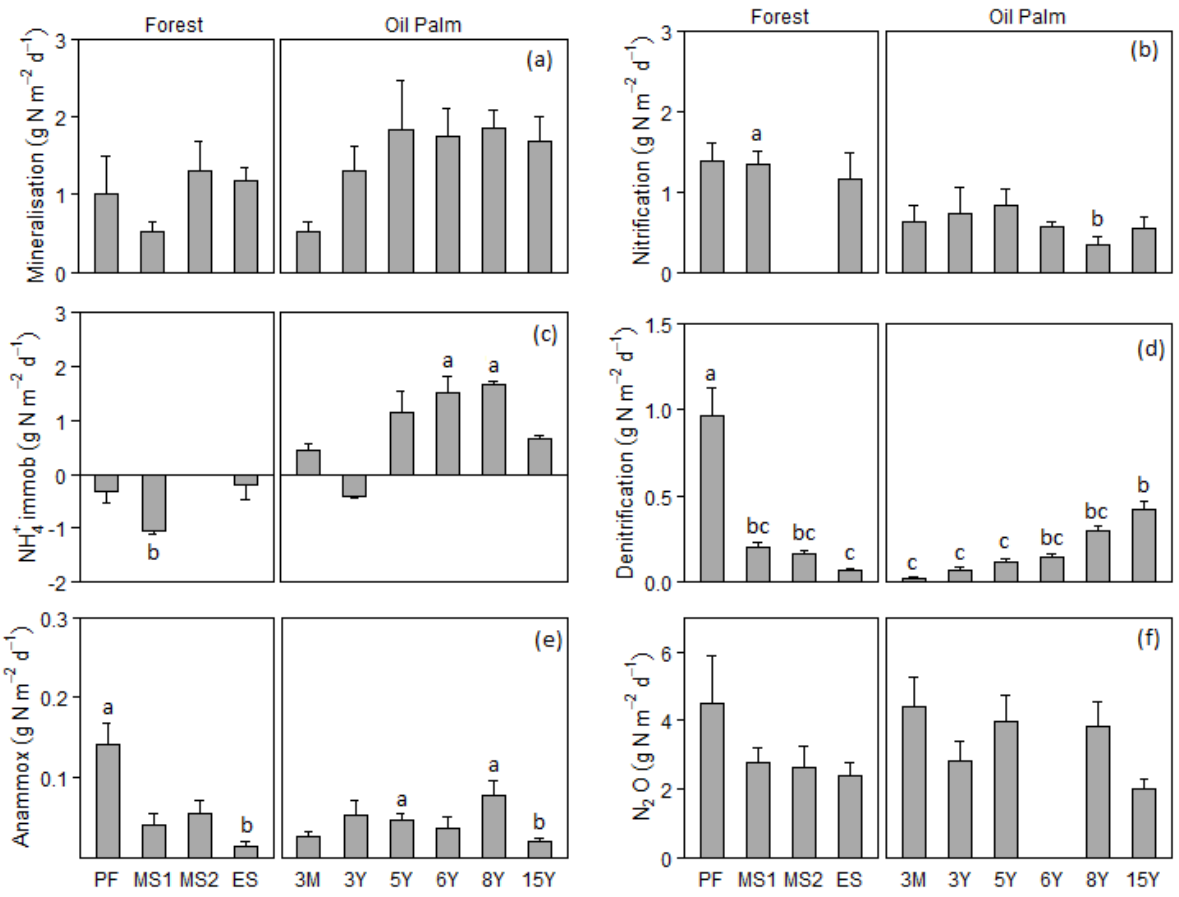

Figure 2: Nitrogen cycling across the chronosequence demonstrates a fundamental alteration to the balance of key nitrogen process rates following forest disturbance (PF - ES) and plantation establishment (3M – 15Y). Rates of gross N mineralisation (a) remain unchanged but a significant decline in gross nitrification (b) and increasing NH_4^+ immobilisation (c) interrupt the pathway to N_2 production via denitrification (d) and "anammox" (e). N_2O production (f) remains unchanged across the chronosequence potentially resulting in high ratios of N_2O:N_2 emission immediately following soil disturbance (see Fig. 3). Different lowercase letters represent significant differences between sites (one-way ANOVA, Tukey multiple comparison of means at $p > 0.05$). Bars represent means ±SE.

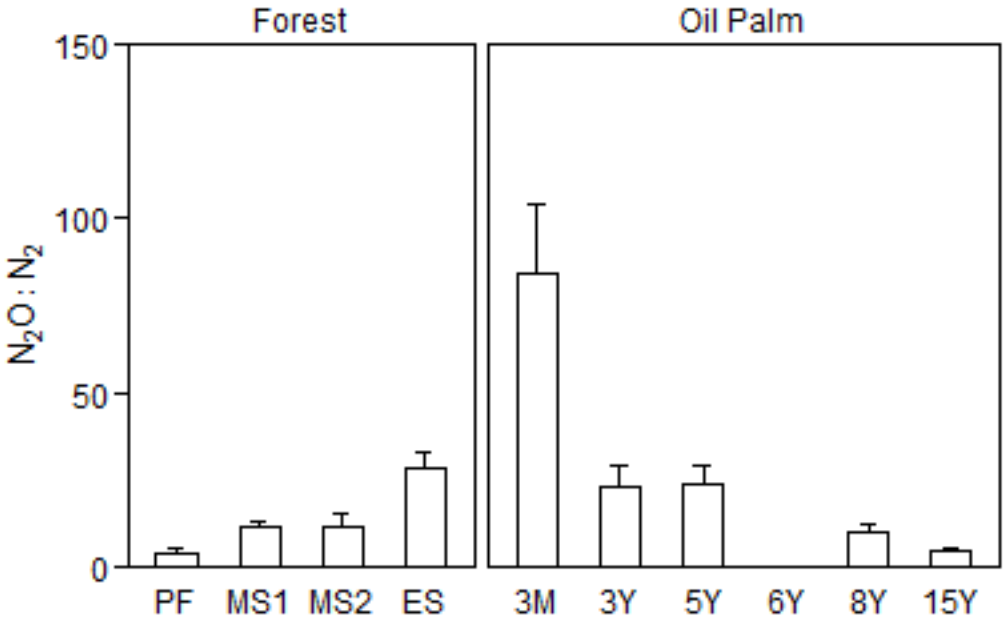

Figure 3: The ratio of N_2O:N_2 production in forest (left) and plantations (right). The decline in N_2 production (as the sum of denitrification and "anammox") increases the potential for N_2O emission in recently disturbed soils.

Table 1: Soil physical and chemical properties for the ten study sites located in Lower Kinabatangan. Values reported are means with standard error in parenthesis. Different lowercase letters indicate significant differences between the sites across both land uses.

Site	Organic matter (%)		Total N (%)		C:N		NO_3^- (g N m^{-2})		NH_4^+ (g N m^{-2})		pH		Bulk density (g cm^{-3})		Sand (%)		Clay (%)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Forests																		
PF	15.13	(2.28)a	0.40	(0.06)a	9.77	(0.40)	0.19	(0.03)	0.66	(0.10)	5.10	(0.10)b	0.87	(0.04)b	25.27	(0.95)a	24.12	(0.45)bc
MS1	7.41	(0.89)	0.30	(0.03)ab	11.06	(0.68)a	0.19	(0.02)	1.09	(0.11)	5.09	(0.11)b	1.06	(0.04)	14.88	(0.56)	25.45	(0.34)
MS2	6.18	(0.64)b	0.17	(0.02)c	10.11	(0.31)	0.11	(0.02)b	0.67	(0.08)	5.16	(0.13)b	0.91	(0.02)b	27.82	(1.80)ab	20.61	(0.50)cd
ES	5.38	(0.53)b	0.19	(0.01)bc	10.66	(0.64)a	0.21	(0.03)	2.02	(0.47)a	4.94	(0.08)bc	1.11	(0.02)	20.43	(0.45)	24.97	(0.33)bcd
Plantations																		
3M	4.86	(2.03)c	0.10	(0.01)d	10.13	(0.68)	0.23	(0.05)	2.42	(0.77)ab	5.18	(0.11)b	1.17	(0.05)	16.89	(0.69)	27.64	(0.35)b
3Y	7.76	(0.62)ab	0.24	(0.02)abc	9.57	(0.60)	0.22	(0.02)a	0.48	(0.06)c	4.41	(0.06)cd	0.94	(0.02)b	19.91	(0.45)ab	26.19	(0.39)
5Y	15.20	(1.83)a	0.30	(0.05)abc	10.11	(0.44)	0.15	(0.02)	0.85	(0.08)	5.91	(0.23)a	1.08	(0.03)	11.96	(1.19)b	31.16	(1.13)a
6Y	6.13	(0.25)bc	0.19	(0.02)bc	8.46	(0.19)b	0.29	(0.04)a	0.91	(0.29)b	4.47	(0.06)cd	1.29	(0.02)a	8.06	(0.84)c	30.32	(0.46)b
8Y	7.79	(1.17)	0.32	(0.04)ab	10.72	(0.49)a	0.18	(0.02)	0.83	(0.06)	4.88	(0.20)bcd	1.14	(0.03)	22.24	(0.96)ab	22.63	(0.81)cd
15Y	8.62	(1.47)ab	0.18	(0.01)bc	10.52	(0.57)a	0.43	(0.20)a	0.57	(0.05)b	4.34	(0.07)d	1.10	(0.01)	7.01	(0.47)c	29.12	(0.52)b