**Cover crops affect the partial nitrogen balance in a maize-forage cropping system**

**ABSTRACT**

Part of the nitrogen (N) fertilizer applied to crops is lost to the environment, contributing to global warming, eutrophication, and groundwater contamination. However, low N supply stimulates soil organic N turnover and carbon (C) loss, since the soil N/C ratio in soil is quasi-constant, ultimately resulting in land degradation. Grasses such as ruzigrass (*Urochloa ruziziensis*) grown as winter pasture or a cover crop in rotation with maize (*Zea mays*) can reduce N leaching, however, this may induce N deficiency and depress yields in the subsequent maize crop. Despite the potential to decrease N loss, this rotation may negatively affect the overall N balance of the cropping system. However, this remains poorly quantified. To test this hypothesis, maize, fertilized with zero to 210 kg N ha$^{-1}$, was grown after ruzigrass, palisade grass (*Urochloa brizanta*) and Guinea grass (*Pannicum maximum*), and the N inputs, outputs and partial N balance determined. Despite the intrinsically poor soil quality associated with the tropical Ultisol, maize grown after the grasses was efficient in acquiring N, resulting in a negative N balance even when 210 kg ha$^{-1}$ of N was applied after Guinea grass. Losses by leaching, N$_2$O emission and NH$_3$ volatilization did not exceed 13.8 kg ha$^{-1}$ irrespective of the grass type. Despite a similar N loss among grasses, Guinea grass resulted in a higher N export in the maize grain due to a higher yield, resulting in a more negative N balance. Soil N depletion can lead to C loss, which can result in land degradation.

**Keywords:** Cropping system; tropical forage; Nitrogen balance; Nitrogen loss; Nitrogen volatilization.

**Introduction**

The progressive increase in agricultural yields seen in many regions of the world in recent decades has been attributed to N fertilization, however, a considerable proportion of this N (ca. 40-60%) is subsequently lost to the environment (Rosolem et al., 2017), which, besides being costly to farmers, may worsen global warming and induce eutrophication (Shelton et al., 2018). Since the soil N/C ratio is approximately constant (Raphael et al., 2016), N loss also implies a concomitant loss of soil C.

Nitrate and dissolved organic N leaching, ammonia ($NH_3$) volatilization from fertilizers and plant tissues, and denitrification ($N_2 + N_2O$) are globally considered to be the main soil N loss pathways, besides soil erosion. However, the amount of N lost as $N_2$ from fertilizers and manures applied to agricultural lands is rarely quantified, due to the difficulties in measuring $N_2$ emissions directly (Zaman et al., 2012). In Brazilian arable cropping regimes, losses from nitrate leaching can be appreciable, reaching up to 87 kg N $ha^{-1}$ $y^{-1}$ (Villalba et al., 2014). Similarly, large gaseous losses of $N-NH_3$ may occur especially with some fertilizer types (e.g. urea, manures) in high pH soils or from stomatal emission from leaves of crops and grasses (Farquhar et al., 1980; Franco et al., 2008). Appreciable loss of N as gaseous $NH_3$ may also occur after crop desiccation, and it has been shown that this loss increases with the amount of N accumulated in plant tissue (Castoldi et al., 2014). In field grown maize nearly 15% of the applied $^{15}N$ was lost through the leaves as $NH_3$ (Francis et al., 1997). Under conditions of high-humidity where $N-NO_3^-$ leaching is limited, denitrification may occur (Subbarao et al., 2013). Typically, denitrification losses increase with N fertilizer application rates with an average $N_2O:N_2$ production ratio of 1:1 (Wang et al., 2018). In well-drained agricultural soils, however, from a plant nutrition perspective $N-N_2O$ losses are low, ranging from 0.20 to 0.52 g $ha^{-1}$ $d^{-1}$ in pastures with Guinea grass (*Panicum maximum*) and palisade grass (Carmo et al., 2005). Although $N-N_2O$ emissions from crop residues may occur after harvest, the flux is assumed to

51  be very low in comparison with organic wastes applied to soil (e.g. manure, slurry, compost;

52  Charles et al., 2017). In terms of crop N offtake, maize grains contain ca. 10% crude protein

53  and therefore N export in grains can be estimated at 16 kg N $t^{-1}$ (Oliveira et al., 2004). Forage

54  grasses do not have a defined export value of N in rotational cropping systems, however, when

55  they are used as cover crops none is exported.

56        Biological fixation of atmospheric N (BNF) can be an important means of N entry into

57  agricultural systems, mainly when legumes are present. Nevertheless, the contribution of

58  grasses may also be important, as it has been reported that BNF accounted for 30 to 40% of the

59  N in humidicola (*U. humidicola*) and signal grass (*U. decumbens*) (Boddey and Victoria, 1986).

60  N can also enter the system in the form of oxides, deposited by rain or dust, or from electric

61  discharge, although these levels are typically considered to be low (4.2 to 6.0 kg N $ha^{-1}$ $y^{-1}$;

62  Shaw et al., 1989). However, in intensive production systems with high productivity, the

63  addition of N fertilizer will remain necessary for a foreseeable future.

64        It has been suggested that the introduction of deep rooted cover crops with vigorous

65  early shoot and root growth offers one potential tool for reducing N losses and enhancing NUE

66  in cropping systems (Rosolem et al., 2018; Shelton et al., 2018). Another potential mitigation

67  strategy is the use of plant species that release chemical compounds that stimulate or suppress

68  the activity of nitrifying microorganisms in the rhizosphere. High rates of biological

69  nitrification inhibition (BNI) have been found in tropical pasture grasses, mainly *Urochloa* spp,

70  which are adapted to growing in low-N environments (Subbarao et al., 2007; Subbarao et al.

71  2009). However, it has been shown that BNI by *Urochloa* spp and decreased N loss is not as

72  evident in N-rich environments (Castoldi et al., 2013), and that BNI-activity is relatively weak

73  in species adapted to high-N such as *Panicum* spp (Subbarao et al., 2015).

74        Forage grasses with vigorous root systems have been widely used in tropical and

75  subtropical regions in integrated cropping systems as winter pastures or as cover crops. Usually,

76 forage grasses, mainly ruzigrass, are grown in consortium or in rotation with maize or soybean

77 (Soratto et al., 2011). However, N deficiency symptoms and decreased maize yields have been

78 observed when it is grown after ruzigrass (Marques, 2015) compared with palisade grass.

79 Therefore, we hypothesized that growing forage grasses as cover crops in rotation with maize

80 has a potential to decrease N loss from the system, but can also impair N uptake, eventually

81 affecting the partial N balance in the cropping system. Furthermore, an over exploitation of soil

82 N may lead to land degradation, which has not been previously studied in tropical agricultural

83 systems. The choice of forage species with optimal exploration of the soil profile to capture N,

84 and a high NUE is very important for sustainability. The objective of this work was to evaluate

85 the effect of ruzigrass, palisade grass and guinea grass on N input, output and partial balance in

86 a cropping system where maize was grown after these species.

87

88 **Materials and Methods**

89     A field experiment was conducted in Botucatu, State of São Paulo, Brazil, 22º 49'

90 27.58" S and 48º 25' 46.73" W, with an altitude of 770 m above sea level, for two seasons. The

91 soil is a Typic Rhodudult (USDA, 2010), with clay texture (626 g clay kg$^{-1}$) and less than 3%

92 slope. The climate is Csa, humid subtropical, according to the Köppen classification (Peel et

93 al., 2007), with an average annual rainfall of 1,400 mm, average temperature of the hottest

94 month exceeding 22ºC and of the coldest month between 3 and 18ºC. The climatic data during

95 the experiment were recorded in a meteorological station located about 500 m from the

96 experiment (Fig. 1). Prior to use, the experimental area had been fallow for four years and was

97 covered by pioneer plants, primarily grass weeds. Soil samples were taken from the topsoil (0

98 to 20 cm) prior to the experiment commencing (Table 1).

99    The experimental design was a complete randomized blocks with subplots and four

100   replicates. The grass forages ruzigrass (*Urochloa ruziziensis*, cv. Common), palisade grass

101   (*Urochloa brizantha*, cv. Marandú) and Guinea grass (*Panicum maximum*, cv. Tanzania) were

102   grown in the plots. After grass desiccation the residues were left on the soil surface, and maize

103   (*Zea mays* L.) was planted in subplots with N added at rates of 0, 70, 140 and 210 kg N ha$^{-1}$, as

104   ammonium sulphate over the grass residues.

105   The experimental area was desiccated with 1.8 kg ha$^{-1}$ glyphosate (a.i.) prior to direct

106   (no till) forage seeding. Forage grasses were planted over the residue straw without fertilizers

107   in rows 0.2 m apart, at a density of 10 kg ha$^{-1}$ of live seeds. Plots were 9.0 m wide $\times$ 20.0 m

108   long. Grasses were grown for 11 months and then desiccated with 2.8 kg ha$^{-1}$ (a.i.) glyphosate

109   and 0.6 kg ha$^{-1}$ (a.i.) of paraquat + 0.3 kg ha$^{-1}$ (a.i.) diuron. After 11 days, maize was planted

110   over the grass residues, using the hybrid 2B810 (Dow Agroscience), in rows 0.75 m apart, with

111   a plant population of 65,000 plants ha$^{-1}$. At planting, 100 kg ha$^{-1}$ of K and 52 kg ha$^{-1}$ of P were

112   applied as potassium chloride and triple superphosphate, respectively, beside and below the

113   seeds, plus 30 kg ha$^{-1}$ of N as ammonium sulphate, banded on plots receiving N. The balance

114   of N fertilizer, i.e., 40, 110 and 180 kg ha$^{-1}$ was applied to the respective treatments when the

115   plants were at the V4-V5 stage, 25 days after sowing, banded 0.2 m next to the sowing line.

116   Crop management (weed, pest and disease monitoring) was undertaken in accordance with

117   standard farm practice and the maize was harvested 133 days after planting.

118   Nitrogen addition by fertilizer to the plots was as described above. The input of rainfall

119   N was quantified by assessing the amount and N concentration in rainwater. Two rain gauges

120   were installed in the experimental area, and immediately after each rainfall event, samples were

121   collected, stored in glass vials and refrigerated until analysis. The nitrate content was quantified

122   using ultraviolet spectroscopy (Eaton et al., 1993) and ammonium colorimetrically by the

salicylic blue method (Qiu et al., 1987), both using an UVmini-1240 spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan).

Water drainage below 0.8 m depth was estimated by monitoring soil moisture with Diviner 2000® capacitance probes (Sentek, Adelaide, Australia). Access tubes were installed in each plot to 1.0 m deep, and soil moisture measurements were taken weekly at 0.1 m depth intervals, both for forage grasses and maize. Water was considered drained when it exceeded the point of saturation in all the soil profile. Water drainage was estimated with the aid of IrriMax® software (Sentek, Adelaide, Australia). Total drainage below 0.80 m was estimated by integrating the amount of water drained at each sampling time. To determine the nitrate content in the soil solution, porous cups were placed at a depth of 0.8 m in the soil. Immediately after rainfall events, a vacuum was applied to the cups and soil solution samples were collected 2 to 3 days after rainfall. The samples were frozen at -15 $^{\circ}$C until analysis. The soil solution $NO_3^-$ content was determined by ultraviolet absorption spectrophotometry as described above. Leaching was estimated by interpolating and multiplying the solution $NO_3^-$ concentration by the volume of drained water in each period and by integrating the results.

The emission of $N_2O$ was quantified using static chambers with a diameter of 30 cm in plots with the forage grass species, and maize fertilized with 0 and 140 kg ha$^{-1}$ N. Headspace air samples were collected 0, 5, 10 and 20 min after closing the chambers, with 20 ml nylon syringes (Bowden et al., 1990). $N_2O$ concentrations were determined by gas chromatography (Shimadzu – GC 2014, Greenhouse model, Shimadzu Scientific Instruments, Kyoto, Japan). At the time of sampling, soil temperature and soil moisture were determined. The date crops were planted were taken as a reference for scheduling gas collection (La Scala Junior et al., 2009). Thus, gas samples were collected at 1, 3, 5, 8, 15, 30 and 60 days after sowing and desiccation of the grasses and after maize sowing, always between 08:00 h and 10:00 h. In other periods of the year, samples were taken monthly. The results were scaled up to one hectare.

148        Volatilization of $NH_3$ was quantified using open collectors with foam absorbers

149    (Oliveira et al., 2008). Each absorber was composed of a foam and acrylic plate. The foam was

150    $8 \times 8$ cm, with a density of 20 kg m$^{-3}$ and was soaked with 11 mL of a 0.5% phosphoric acid +

151    4% glycerol solution. The foam was placed on $10 \times 10 \times 0.2$ cm acrylic plates and surrounded

152    by a layer of polytetrafluoroethylene tape, which is permeable to $NH_3$ and impermeable to

153    water. Until placed in the field, the absorbers were stored in plastic bags and refrigerated to

154    avoid contamination. The collectors were placed just over the plant canopies, and adjusted as

155    needed. During the forage phase of the experiment, the foam was replaced every two weeks in

156    the first month and monthly until desiccation. After desiccation, a collection cycle was

157    performed for 20 days, spaced every four days. In maize, two cycles of 15-day intervals were

158    carried out five days after sowing and after N fertilization. In other periods of the cropping

159    cycle, samples were collected monthly. After collection, the foam was placed in sealed

160    containers and refrigerated at 4 $^{\circ}$C or analyzed immediately. For analysis, samples were washed

161    with 100 mL of 0.0005 N sulfuric acid, and an aliquot was analyzed in duplicate by the salicylic

162    blue method (Qiu et al., 1987), in a UVmini-1240 spectrophotometer (Shimadzu Scientific

163    Instruments, Kyoto, Japan). The amount of $NH_3$ captured in the traps was extrapolated to one

164    hectare.

165        Forage grasses were cut 0.3 m above soil level 6 and 8 months after sowing. The cut

166    material was removed from the plot, and considered exported material. The exported dry matter

167    was estimated by randomly taking three 0.25 m$^2$ sub-samples per plot. The sampled material

168    was oven-dried to constant weight at 65 $^{\circ}$C and weighed. A sub-sample was used to determine

169    its N content. Maize grain yield was determined by harvesting 6.0 m of the three central rows,

170    totaling 13.5 m$^2$. Yield was estimated at a grain moisture of 13%. A grain subsample was taken

171    to determine the N concentration in the tissue. Tissue N was analyzed after acid digestion, by

172    distillation with sodium hydroxide and subsequent titration with 0.1 N sulfuric acid.

173    The partial N balance in the system comprised the whole period of forage grasses (11

174    months) and maize cropped in succession (5 months), totaling 16 months, and was calculated

175    according to the following equation (Equation 1):

176

177

178    (1)                    $NBal = (raN + feN) - (NO_3^- + NH_3 + N_2O + grN + maN)$

179

180

181    where: NBal = N balance; raN = N from rain; feN = N from fertilizer; $NO_3^-$ = N loss by nitrate

182    leaching; $NH_3$ = N loss by ammonia volatilization; $N_2O$ = N loss by nitrous oxide emission;

183    grN = N exported via grasses; maN = N exported via maize.

184

185

186    Data were submitted to analysis of homogeneity of variance (Levene test) and normality

187    (Shapiro-Wilk). Based on these assumptions, ANOVA was performed ($p<0.05$) considering a

188    sub-plot model, with three treatment (grasses) in plots and four N rates in sub-plots, with four

189    replicates. When significant, means of the species were compared by the Tukey test ($p<0.05$),

190    and the response to N rates was submitted to regression analysis; in case of interaction,

191    regression of N rates was calculated for each species. The SAS software version 9.2 was used

192    for all the analysis (SAS Institute Inc., 2009).

193

194    **Results**

195    The total rainfall during the 16-month study period was 3,316 mm (Figure 1), giving a

196    total wet deposition of 5.8 kg ha$^{-1}$ of N in the production system, 3.0 kg ha$^{-1}$ of N during the

197    forage cycle and 2.7 kg ha$^{-1}$ of N during the maize crop. Nitrogen input via fertilizers was

198     controlled by applying the fertilizer under adequate conditions of humidity and soil moisture as

199     described earlier.

200          Nitrogen losses such as $NO_3^-$ leaching, $N_2O$ emissions, and $NH_3$ volatilization did not

201     exceed 14 kg ha$^{-1}$ in total, with no significant differences observed between treatments (Figure

202     2). The quantities of $N$-$NO_3^-$ and $N$-$N_2O$ lost were ca. 1.0 kg ha$^{-1}$ throughout the growing season

203     of forage grasses and maize, regardless of grass species and N application rate. The

204     volatilization of $N$-$NH_3$ contributed, on average, ca. 12 kg ha$^{-1}$ of N.

205          The sum of N exported by forage grasses was 50, 49 and 50 kg ha$^{-1}$ of N for ruzigrass,

206     palisade grass and Guinea grass, respectively. Nitrogen export in maize grains was affected by

207     N rates and forage species, with no interaction of the factors (Table 2). Nitrogen was exported

208     in greater quantities by maize cropped after Guinea grass than after palisade grass and ruzigrass.

209     Overall, the N export increased with fertilizer rates. In the absence of N, maize exported a little

210     more than 30 kg ha$^{-1}$ of N, while the average N export reached 159 kg ha$^{-1}$ with the application

211     of 210 kg ha$^{-1}$ of N.

212          In summary, the partial N balance in the production system was strongly affected by

213     both forage grasses and fertilizer application rate, with interactions between these two factors

214     (Figure 3 and Table3). The balance was negative in almost all treatments except for 210 kg ha$^-$

215     $^1$ of N after palisade grass. The production system with Guinea grass had the most negative

216     balance, removing 21 kg ha$^{-1}$ more N than was added to the system at the rate of 210 kg ha$^{-1}$ of

217     N. Without N fertilizer, the deficit was 94 kg ha$^{-1}$. Ruzigrass and palisade grass were similar as

218     to the N balance, and in the absence of N both resulted in soil N depletion of around 85 kg ha$^-$

219     $^1$. At the highest N rate applied to maize, the partial balance with ruzigrass and palisade grass

220     was practically nil. On average, the production system with Guinea grass resulted in a higher N

221     deficiency in relation to the systems with ruzigrass and palisade grass. It was also observed that

222     the higher N rate resulted in a less negative N balance as compared with the treatment without

223     N.

224

225     **Discussion**

226          In our study we did not quantify $N_2$ losses by denitrification. Although $N_2$ losses can

227     be high under waterlogged conditions they are generally low in well drained, moderately

228     acidic soils (Zaman et al., 2007) such as the one employed here. As $N_2$ produced in

229     denitrification is frequently correlated with $N-N_2O$ emissions we assume here that they were

230     low based on our measured $N_2O$ fluxes.

231          The deposition of 5.8 kg ha$^{-1}$ of N in 16 months corresponds to around 4.3 kg ha$^{-1}$ year$^{-1}$

232     $^1$ of N added by rainfall, similar to the 4.2 kg ha$^{-1}$ year$^{-1}$ observed in Canada (Shaw et al., 1989).

233     From a plant nutrition perspective, this is low, since it only represents 3.4% of the N

234     requirement for maize yielding from 10-12 t ha$^{-1}$ (Raij et al., 1996). Despite the high amount of

235     rain accumulation during the experiment, N concentration in the water was very low, resulting

236     in a small N input to the production system.

237          Fertilizer was the main N input in our system. Ammonium sulphate, the N source used

238     in the present experiment, is not conducive to $N-NH_3$ volatilization (Chambers and Dampney,

239     2009), which would only occur under soil alkalinity (Volk, 1959). However, ammonium added

240     to the soil via fertilizer may undergo nitrification, and become susceptible to leaching.

241          Losses due to $N-NO_3^-$ leaching and $N-N_2O$ emissions, although small in the present

242     study and usually less than 1.0 kg ha$^{-1}$, have been strongly debated in the literature considering

243     their potential for environmental damage. Nitrate leaching in sandy soils can contaminate the

244     water table and $N-N_2O$ is an important greenhouse gas. Losses by $N-N_2O$ emission are typically

245     low in tropical regions, below 1.0 kg ha$^{-1}$ year$^{-1}$ in a Ultisol with grazed pastures of Guinea

246     grass and palisade grass (Carmo et al., 2005). Nitrate leaching above 17 kg ha$^{-1}$ was observed

247   in a production system with soybean, crotalaria (*Crotalaria spectabilis*), millet (*Pennisetum*

248   *americanum*) and sorghum (*Sorghum bicolor*), without addition of N fertilizer (Rosolem et al.,

249   2018), and N loss strongly correlated with the amount of N present in the soil profile, since

250   systems with more legumes resulted in higher losses. N leaching is also frequently related with

251   the rainfall amount, which was high during the present experiment (Fig 1). However, losses of

252   less than 5% of the fertilizer N were observed in Brazilian Ultisols with rates from 100 to 120

253   kg ha$^{-1}$ of N (Ghiberto et al., 2009; 2015), which were not related with rainfall (Rosolem et al.,

254   2017). In the present study, the deep-rooted grasses had probably depleted soil N in the profile,

255   avoiding leaching (Rosolem et al., 2017). In addition, maize roots can reach depths of up to 1.5

256   m (Ordóñez et al., 2018) and the demand for N is high, which may explain the low N-NO$_3^-$

257   leaching losses, even with fertilizer rates up to 210 kg ha$^{-1}$ of N.

258        Volatilization of NH$_3$, despite being 12 times greater than N-NO$_3^-$ leaching and N-N$_2$O

259   emission together, was similar for all the forage grasses and was not affected by fertilizer

260   application rate. An important part of N-NH$_3$ volatilization observed here was probably derived

261   from senescing plants after grass desiccation and at the end of maize cycle. These losses were

262   reported to be proportional to the amount of N accumulated in plant tissue (Francis et al., 1997;

263   Castoldi et al., 2014), and were estimated to range from 11 to 17 kg ha$^{-1}$ in palisade grass and

264   grain sorghum in rotation with soybean, in a N-rich production system (Castoldi et al., 2014).

265   Stomatal emission of NH$_3$ from live plant leaves (Farquar et al., 1980) were reported to account

266   for nearly 15% of the applied fertilizer (Francis et al., 1997). In the present study, no fertilizer

267   was applied to grasses, but in maize, even with N rates up to 210 kg ha$^{-1}$ N, N-NH$_3$ losses did

268   not increase, probably because there was no N excess in the system, since the response of maize

269   to N was linear (Table 2).

270        On average, the amount of N exported in maize grains was higher after Guinea grass

271   than after the other grasses. This grass species is adapted to fertile soils, and accumulated 68.0

272     kg ha$^{-1}$ of N by the time of desiccation, against 61.1 and 29.7 kg ha$^{-1}$ accumulated by palisade

273     grass and ruzigrass ($p<0.05$), respectively. This N was not taken out from the field and was not

274     considered in the partial balance, but it was available to maize as the grass residues decayed.

275        Root exudates and rates of root turnover in Guinea grass are also likely to be different

276     from *Urochloa* spp. For example, Guinea grass has been shown to have relatively weak BNI-

277     activity (Subbarao et al., 2015). In contrast, root exudates of *Urochloas* can reduce nitrification,

278     altering the soil N dynamics compared with Guinea grass (Subbarao et al., 2009). The different

279     amounts of N in grass residues could explain the higher export compared with ruzigrass, but

280     does not explain the difference of Guinea grass compared with palisade grass. The lower N

281     accumulation and export in maize grains observed after *Urochloa* compared with Guinea grass

282     may result from the effect of *Urochloa* on soil N dynamics, impairing maize N uptake. It has

283     been hypothesized that the nutrient cycling from the straw to the soil does not occur in a timely

284     manner for maize (Rosolem et al., 2017). However, this may not be the case in this experiment,

285     at least for Guinea grass.

286        The almost-zero balance of the production system with ruzigrass and palisade grass at

287     the rate of 210 kg ha$^{-1}$ of N showed that they left less N available for maize, which resulted in

288     lower N accumulation and export. The negative balances found in the absence of N for

289     ruzigrass, palisade and Guinea grass may be evidence of another N input in the production

290     system. Biological nitrogen fixation, not considered in this work, could be important here, since

291     about 30 to 40% of the N in plants of humidicola and signal grass could have come from BNF

292     (Boddey and Victoria, 1986); in the conditions of the present study, this would represent 30 to

293     45 kg N ha$^{-1}$ year$^{-1}$ in fertilized systems. In the tissues of ruzigrass and palisade grass, N from

294     BNF may be as high a 20 % (Reis et al., 2001), and in a test with 25 genotypes of Guinea grass,

295     it was observed that they could acquire 16 to 49% of N by BNF (Miranda et al., 1990). A

296     contribution of BNF around 35-40 kg ha$^{-1}$ year$^{-1}$ in cultivated pastures (Boddey and Victoria,

297    1986), in addition to rainfall, would represent around 45 kg ha$^{-1}$ of N in our experiment, a

298    significant contribution to meet maize demand, even with legumes in the system. Despite being

299    seasonal, BNF could have occurred, adding N to the system, but it would unlikely to be the

300    same for all three species. Therefore, further studies of BNF in production systems involving

301    grasses of the genus *Urochola* spp and *Pannicum* spp are needed for a better understanding of

302    the N cycling in this kind of agroecosystem.

303      The most negative balance, observed in the production system with Guinea grass, is a

304    result of the higher grain yield, since the greatest differences in the amount of exported N were

305    in the grains (Table 2). The mineralization of N from the grass straw can also be a factor

306    affecting maize yield. The N content and specific characteristics of each grass, such as lignin/N

307    ratio, for example, can determine the rate of straw N release. Thus, N-release may not be

308    synchronized with N-requirement by maize (Rosolem et al., 2017), depending on the grass

309    species. The lignin/N ratio of the forage was on average 1.4, regardless of the N rate, in an N-

310    rich soil with maize intercropped with palisade grass and ruzigrass (Costa et al., 2014).

311    However, the lignin/N ratio was reduced from 5 to 2 with the addition of 400 kg ha$^{-1}$ of N to

312    maize intercropped with Guinea grass, ruzigrass and palisade grass, and the N release time is

313    reduced with low lignin/N ratio straw (Pariz et al., 2011).

314      A negative balance in the production system represents a deficit of N for the crops in

315    succession, and it is necessary to correct for this deficit according to the requirement of the crop

316    to be grown. Therefore, a high productivity system with a negative balance will have a greater

317    need for adding N to supply the deficit, requiring more investment. If the most negative balance

318    is caused by losses, such as leaching of N-NO$_3^-$, volatilization of N-NH$_3$ and emission of N-

319    N$_2$O, mitigation should be recommended. The use of specific species can help reduce loss

320    (Byrnes et al., 2017), by using humidicola and a hybrid called Mulato (*U. ruziziensis* $\times$ *U.*

321    *brizantha* cv. Marandú), which successfully reduced nitrification of N-NH$_4^+$ from cattle urine

322    in pasture areas, resulting in lower losses by denitrification. However, this was not the case in

323    the present study. The highest peak of N losses due to volatilization of $N-NH_3$ occurs shortly

324    after desiccation of the cover crops (Castoldi et al., 2014), but in the present experiment this

325    was not sufficient to explain the N deficiency in the system.

326    The N export was linear as a function of the applied N rates. In this sense, it is

327    important to acknowledge the very high NUE in the production systems of the present

328    experiment. About 70 to 90% of the N applied via fertilizer was used by the system, which

329    contrasts strongly with maize forage rotation systems where only 35 % was used by maize

330    (Couto-Vázquez and González-Prieto, 2016). Therefore, it can be inferred that maize was able

331    to use N from the soil and from the mineralization of the forage straw, besides the N provided

332    by fertilizer. It has also been demonstrated that that 86% of the N acquired by maize grown

333    after humidicola came from the soil (Karwrt et al., 2017).

334    In general, the N balance was negative regardless of the forage grass species or N rate.

335    Thus, N input via BNF could be contributing to N supply, as discussed earlier. No significant

336    N loss was observed in this study, but all the rotations can lead to soil N depletion in time,

337    requiring the application of high N rates to keep the balance with C (Raphael et al., 2016) and

338    be sustainable.

339

340    **Conclusions**

341    When grown after ruzigrass, palisade grass and Guinea grass, maize is efficient in

342    acquiring soil N, even with the application of up to 210 kg ha$^{-1}$ of N. The use of ruzigrass,

343    palisade grass and Guinea grass in rotation with maize in a clay soil result in low and similar N

344    losses by $N-N_2O$ emission and $N-NH_3$ volatilization and do not lose N by $N-NO_3^-$ leaching.

345    However, Guinea grass results in higher N export in maize grains due to a higher yield, resulting

346    in a more negative N balance. A negative N soil balance year after year may in time deplete

347 soil N, and eventually soil C, compromising the sustainability of the system. This result is

348 important because it shows that N under-fertilization may be as harmful for sustainability as

349 over fertilization.

350

358

359 **References**

360 Boddey, R.M., Victoria, R L., 1986. Estimation of biological nitrogen fixation associated with

361 Brachiaria and Paspalum grasses using[15]N labelled organic matter and fertilizer. Plant and Soil,

362 90, 1–3, 265–292. doi: 10.1007/BF02277403

363

364 Bowden, R.D., Steudler, P.A., Melillo, J.M., 1990. Annual nitrous oxide fluxes from temperate

365 forest soil in the northeastern United States. Journal of Geophysical Research, 95, 13997-

366 14005. doi: 10.1029/JD095iD09p13997

367

368 Byrnes, R.C., Núñez, J., Arenas, L., Rao, I., Trujillo, C., Alvarez, C., Arango, J., Rasche, F.,

369 Chirinda, N., 2017. Biological nitrification inhibition by Brachiaria grasses mitigates soil

370 nitrous oxide emissions from bovine urine patches. Soil Biology & Biochemistry, 107, 156-

371 163. doi: 10.1016/j.soilbio.2016.12.029

372

373    Carmo, J.B., Andrade, C.A., Cerri, C.C., Piccolo, M.C., 2005. Disponibilidade de nitrogênio e

374    fluxos de $N_2O$ a partir de solo sob pastagem após aplicação de herbicida. Revista Brasileira de

375    Ciência do Solo, 29, 735-746. doi: 10.1590/S0100-06832005000500009.

376

377    Castoldi, G., Pivetta, L.A., Rosolem, C.A., 2014. Nitrogen budget in the soil-plant system after

378    brachiaria grasses desiccation. Soil Science and Plant Nutrition, 60, 162-172. doi:

379    10.1080/00380768.2013.878641

380    Castoldi, G., Reis, J.G., Pivetta, L.A., Rosolem, C.A., 2013. Soil nitrogen dynamics after

381    brachiaria desiccation. Revista Brasileira de Ciência do Solo, 37, 1620-1627. doi:

382    10.1590/S0100-06832013000600018

383

384    Chambers, B., Dampney, P., 2009. Nitrogen efficiency and ammonia emissions from urea-

385    based and ammonium nitrate fertilisers, in: Proceedings-International Fertiliser Society (No.

386    657). International Fertiliser Society. 20p.

387

388    Charles, A., Rochette, P., Whalen, J.K., Angers, D.A., Chantigny, M.H., Bertrand, N.,2017.

389    Global nitrous oxide emission factors from agricultural soils after addition of organic

390    amendements: A meta-analysis. Agriculture, Ecosystems and Environment, 236, 88-98. doi:

391    10.1016/j.agee.2016.11.021

392

393    Costa, N.R., Andreotti, M., Buzetti, S., Lopes, K.S.M., Santos, F.G., Pariz, C.M., 2014.

394    Acúmulo de macronutrientes e decomposição da palhada de braquiárias em razão da

395    adubação nitrogenada durante e após o consórcio com a cultura do milho. Revista Brasileira

396    de Ciência do Solo, 38, 1223–1233. doi:10.1590/S0100-06832014000400019

397

398 Couto-Vázquez, A., González-Prieto, S.J., 2016. Fate of 15N-fertilizers in the soil-plant system

399 of a forage rotation under conservation and plough tillage. Soil & Tillage Research, 161, 10–

400 18. doi: 10.1016/j.still.2016.02.011

401

402 Eaton, A.D., Clesceri, L.S., Greenberg, A.E., 1993. Standard methods for the examination of

403 water and wastewater (18th ed.), Washington, DC: APHA, AWWA, WEF.

404

405 Farquhar, G.D., Firth, P.M., Wetselaar, R., Weir, B., 1980. On the gaseous exchange of

406 ammonia between leaves and the environment: Determination of the ammonia compensation

407 point. Plant Physiology, 66, 710-714. doi: 10.1104/pp.66.4.710

408

409 Francis, D.D., Schepers, J., Sims, A.L., 1997. Ammonia exchange from corn foliage during

410 reproductive growth. Agronomy Journal, 89, 941-946, doi:

411 10.2134/agronj1997.00021962008900060015x

412

413 Franco, H.C.J., Damin, V., Franco, A., Moraes, M.F., Trivelin, P.C.O., 2008. Perda de

414 nitrogênio pela *Brachiaria decumbens* após a antese: relação com a umidade do solo. Ciência

415 Rural, 38, 96-102. doi: 10.1590/s0103-84782008000100016

416

417 Friedl, J., Scheer, C., Rowlings, D.W., Mumford, M.T., Grace, P.R., 2017. The nitrification

418 inhibitor DMPP (3,4-dimethylpyrazole phosphate) reduces $N_2$ emissions from intensively

419 managed pastures in subtropical Australia. Soil Biology and Biochemistry, 108, 55-64. doi:

420 10.1016/j.soilbio.2017.01.016

421

422 Ghiberto, P. J., Libardi, P. L., Brito, A.S., Trivelin, P.C.O., 2009. Leaching of nutrients from

423 a sugarcane crop growing on an Ultisol in Brazil. Agricultural Water Management, 96, 1443–

424 1448. doi: 10.1016/j.agwat.2009.04.020

425

426 Ghiberto, P.J., Libardi, P.L., Trivelin, P.C.O., 2015. Nutrient leaching in an Ultisol cultivated

427 with sugarcane. Agricultural Water Management., 148, 141–149. doi:

428 10.1016/j.agwat.2014.09.027

429

430 Karwat, H., Moreta, D., Arango, J., Núñez, J., Rao, I., Rincón, Á., Rasche, F., Cadisch, G.,

431 2017. Residual effect of BNI by *Brachiaria humidicola* pasture on nitrogen recovery and grain

432 yield of subsequent maize. Plant and Soil, 420, 389–406. doi: 10.1007/s11104-017-3381-z

433

434 Keeney, D.R., Nelson, D.W., 1982. Nitrogen: inorganic forms, in: Page A.L., Miller, R.H.,

435 Keeney, D.R. Methods of soil analysis (2nd ed.) Madison: American Society of Agronomy.

436

437 La Scala Junior, N., Lopes, A., Spokas, K., Archer, D.W., Reicosky, D., 2009. First-order decay

438 models to describe soil C-CO2 loss after rotary tillage. Scientia Agricola, 66, 650-657. doi:

439 10.1590/S0103-90162009000500010

440

441 Marques, L.M., 2015. Antecipação de nitrogênio para a cultura do milho em sucessão a

442 gramíneas forrageiras no sistema plantio direto. Masters Dissertation, Universidade Estadual

443 Paulista "Júlio de Mesquita Filho" – Faculdade de Ciências Agronômicas de Botucatu, 47p.

444

445    Miranda, C.H.B., Urquiaga, S., Boddey, R.M., 1990. Selection of ecotypes of Panicum

446    maximum for associated biological nitrogen fixation using the $^{15}$N isotope dilution technique.

447    Soil Biology and Biochemistry, 22, 657–663. doi: 10.1016/0038-0717(90)90012-o

448

449    Oliveira, J.P., Chaves, L.J., Duarte, J.B., Brasil, E.M., Ferreira Junior, L.T., Ribeiro, K.O.,

450    2004. Teor de proteína no grão em populações de milho de alta qualidade protéica e seus

451    cruzamentos. Pesquisa Agropecuária Tropical, 34, 45-51. Available in

452    https://www.revistas.ufg.br/pat/article/view/2341.

453

454    Oliveira, P.P.A., Trivelin, P.C.O., Alves, A., Luz, P.H.C., Herling, V.R., 2008. Métodos para

455    avaliar as perdas de nitrogênio por volatilização da superfície do solo e por emissão de amônia

456    pela folhagem de *Brachiaria brizantha cv. Marandu*. São Carlos, Sao Paulo, Brazil. Embrapa

457    Pecuária Sudeste, 41p. (Boletim, 16). Available in https://www.embrapa.br/busca-de-

458    publicacoes/-/publicacao/48446/metodos-para-avaliar-as-perdas-de-nitrogenio-por-

459    volatilizacao-da-superficie-do-solo-e-por-emissao-de-amonia-pela-folhagem-de-brachiaria-

460    brizantha-cv-marandu.

461

462    Ordóñez A. R., Castellano, M.J., Hatfield, J.L., Helmers, M.J., Licht, M.A., Liebman, M.,

463    Dietzel, R., Martinez-Feria R., Iqbal, J., Puntel, L.A., Córdova, S.C., Togliatti, K., Wright,

464    E.E., Archontoulis, S.V., 2018. Maize and soybean root front velocity and maximum depth in

465    Iowa, USA. Field Crops Research, 215, 122-131. *doi:* **10.1016/j.fcr.2017.09.003**

466

467    Pariz, C.M., Andreotti, M., Bergamaschine, A.F., Ulian, N.A., Furlan, L.C., Meirelles, P.R.L.,

468    Cavasano, F.A., 2011. Straw decomposition of nitrogen-fertilized grasses intercropped with

469 irrigated maize in an integrated crop-livestock system. Revista Brasileira de Ciência do Solo,

470 35, 2029–2037. doi: 10.1590/s0100-06832011000600019

471

472 Peel, M.C., Finlayson, B.L., McMahon, T.A., 2007. Updated world map of the Köppen–Geiger

473 climate classification. Hydrol. Earth Syst. Sci. 11, 1633–1644. doi: 10.5194/hess-11-1633-2007

474

475 Qiu, X., Liu, G, Zhu, Y., 1987. Determination of Water-soluble Ammonium Ion in Soil by

476 Spectrophotometry. Analyst, 112, 909-911. doi: 10.1039/AN9871200909

477

478 Raij, B., Quaggio, J.A., Silva, N.M., 1986. Extraction of phosphorus, potassium, calcium, and

479 magnesium from soils by an ion exchange resin procedure. Communications in Soil Science

480 and Plant Analysis, 17, 547-566. doi: 10.1080/00103628609367733

481

482 Raij, B., Cantarella, H., Quaggio, J.A., Furlani, A.M.C., 1996. *Recomendações de adubação e*

483 *calagem para o Estado de São Paulo (*2nd ed.) Campinas, SP: Instituto Agronômico.

484

485 Raij B., Andrade J.C., Cantarella H., Quaggio J.A., 2001. *Análise química para avaliação da*

486 *fertilidade de solos tropicais.* Instituto Agronômico, Campinas, SP, Btrazil.

487

488 Raphael J.P.A, Calonego J.C., Milori D.M.B.P., Rosolem C.A., 2016. Soil organic matter in

489 crop rotations under no-till. Soil and Tillage Research, 155, 45–53, 2016. doi:

490 10.1016/j.still.2015.07.020

491

492  Reis, V.M., Reis Jr., F.B., Quesada, D.M, Oliveira, O.C.A., Alves, B.J R., Urquiaga, S.,

493  Boddey, R.M., 2001. Biological nitrogen fixation associated with tropical pasture grasses.

494  Australian Journal of Plant Physiology, 28, 837–844. doi: 0.1071/PP01079

495

496  Rosolem, C.A., Foloni, J.S.S., Oliveira, R.H., 200). Dinâmica do nitrogênio no solo em razão

497  da calagem e adubação nitrogenada, com palha na superfície. Pesquisa Agropecuária Brasileira,

498  38, 301-309. doi: 10.1590/S0100-204X2003000200018

499

500  Rosolem, C.A., Ritz, K., Cantarella, H., Galdos, M.V., Hawkesford, M.J., Whalley, W.L.,

501  Mooney, S.J., 2017. Enhanced Plant Rooting and Crop System Management for Improved N

502  Use Efficiency. Advances in Agronomy, 205-239. doi: 10.1016/bs.agron.2017.07.002

503

504  Rosolem, C.A., Castoldi, G., Pivetta, L.A., Ochsner, T.E., 2018. Nitrate leaching in soybean

505  rotations without nitrogen fertilizer. Plant and Soil, 423, 27-40. doi: 10.1007/s11104-017-3494-

506  4

507

508  SAS Institute Inc, 2009. The SAS System for Windows. SAS 9.2. SAS Inst., Cary, NC.

509

510  Shaw, R.D., Trimbee, A.M., Minty, H., Fricker, H., Prepas, E.E., 1989. Atmospheric deposition

511  of phosphorus and nitrogen in central Alberta with emphasis in Narrow Lake. Water, Air and

512  Soil Pollution, 43, 119-134.

513

514  Shelton, R., Jacobsen, K.L., McCulley, R., 2018. Cover Crops and Fertilization Alter Nitrogen

515  Loss in Organic and Conventional Conservation Agriculture Systems. Frontiers in Plant

516  Science, 8, 2260. doi: 10.3389/fpls.2017.02260

517

518    Soratto, R.P., Rosolem, C.A., Crusciol, C.A.C., 2011. Integração Lavoura-Pecuária-Floresta.

519    Botucatu, SP, Brazil, FEPAF.

520

521    Subbarao, G.V., Rondon, M., Ito, O., Ishikawa, T., Rao, I.M., Nakahara, K., Lascano, C., Berry,

522    W.L., 2007. Biological nitrification inhibition (BNI) – is it a widespread phenomenon? Plant

523    and Soil, 294, 5-18. doi: 10.1007/s11104-006-9159-3

524

525    Subbarao, G.V., Nakahara, K., Hurtado, M.P., Ono, H., Moreta, D.E., Salcedo, A.F.,

526    Yoshihashi, T., Ishitani, M., Ohnishi-Kameyama, M., Yoshida, M., Rondon, M., Rao, I.M.,

527    Lascano, C.E., Berry, W.L., Ito, O., 2009. Evidence for biological nitrification inhibition in

528    Brachiaria pastures. Proceedings of the National Academy of Science of the United States of

529    America, 106, 17302-17307. doi: 10.1073/pnas.0903694106

530

531    Subbarao, G.V, Rao, I.M., Nakahara, K., Sahrawat, K.L., Ando, Y., Kawashima, T., 2013.

532    Potential for biological nitrification inhibition to reduce nitrification and N2O emissions in

533    pasture crop-livestock systems. Animal: an international journal of animal bioscience, 7, 322–

534    332. doi: 10.1017/S1751731113000761

535

536    Subbarao, G.V., Yoshihashi, T., Worthington, M., Nakahara, K., Ando, Y., Saharawat, K.L.,

537    Rao, I.M., Lata, J., Kishii, M., Braun, H., 2015. Suppression of soil nitrification by plants. Plant

538    Science, 233, 155–164. doi: 10.1016/j.plantsci.2015.01.012

539

540    USDA, United States Department of Agriculture (2010). Keys to Soil Taxonomy (11th ed.),

541    Washington, USDA

542

543 Villalba, H.A.G., Leite, J.M., Otto, R., Trivellin, P.C.O., 2014. Fertilizantes nitrogenados:

544 novas tecnologias. Informações Agronômicas, 148, 12–18. http://www.ipni.net/publication/ ia-

545 brasil.nsf/issue/IA-BRASIL-2014-148

546

547 Vitti, A.C., Trivelin, P.C.O., Gava, G.J.D.C., Franco, H.C.J., Bologna, I.R., Faroni, C.E., 2007.

548 Produtividade da cana-de-açúcar relacionada à localização de adubos nitrogenados aplicados

549 sobre os resíduos culturais em canavial sem queima. Revista Brasileira de Ciência do Solo, 31,

550 491–498. doi: 10.1590/S0100-06832007000300009

551

552 Volk, G., 1959). Volatile loss of ammonia following surface application of urea to turf or bare

553 soil. Agronomy Journal, 51, 746-749. doi: 10.2134/agronj1959.00021962005100120016x

554

555 Wang, J., Chadwick, D.R., Cheng, Y., Yan, X., 2018. Global analysis of agricultural soil

556 denitrification in response to fertilizer nitrogen. Science of the Total Environment, 616, 908-

557 917. doi: 10.1016/j.scitotenv.2017.10.229

558

559 Zaman, M. Nguyen, M.L. Matheson, F., Blennerhassett, J.D., Quin, B.F., 2007. Can soil

560 amendments zeolite or lime shift the balance between nitrous oxide and dinitrogen

561 emissions from pasture and wetland soils receiving urine or urea-N? Australian Journal of

562 Soil Research, 45, 543-553. doi: 10.1071/sr07034

563

564 Zaman, M., Nguyen, M.L., Šimek, M., Nawaz, S., Khan, M.J., Babar, M.N., Zaman, S., 2012.

565 Emissions of nitrous oxide ($N_2O$) and di-nitrogen ($N_2$) from the agricultural landscapes,

566 sources, sinks, and factors affecting N2O and N2 ratios, in: Liu, G. Greenhouse gases-

567 emission, measurement and management. Rijeka, Croatia: InTech.

568

569

570

571 **Table 1.** Selected soil chemical characteristics before the experimental start in the layer of 0.0
572 – 0.2 m depth, and total N in the soil profile to the depth of 0.80 m.

| SOM | pH | $N-NH_4^+$ | $N-NO_3^-$ | P (resin) | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | H+Al | V |
|------|------|------|------|------|------|------|------|------|------|
| g dm$^{-3}$ | CaCl$_2$ | ---------- mg Kg$^{-3}$ ------------ | | | -------------mmol$_c$ dm$^{-3}$--------- | | | | % |
| 20 | 5.9 | 5.4 | 6.3 | 15 | 1.2 | 34 | 23 | 37 | 60 |

| | | | Soil depth | | | |
|------|------|------|------|------|------|------|
| | 0.0-0.1 | 0.1-0.2 | 0.2-0.4 | 0.4-0.6 | 0.6-0.8 | Total |
| | | | Mg ha$^{-1}$ | | | |
| Total N | 1.4 | 1.3 | 2.2 | 1.6 | 1.5 | 8.0 |

573 SOM = (Walkley and Black, 1934); pH and H+Al = (Raij et al., 2001); P, Ca$^{+2}$, Mg$^{+2}$ and K$^+$ = (Raij et
574 al., 1986); N-NH$_4^+$ and N-NO$_3^-$ = (Keeney and Nelson, 1982); Total N = .

575

576

577

578

579

580

581

582

583 **Table 2.** Average (n=4) amount of nitrogen exported via maize grains cropped after ruzigrass,
584 palisade grass and Guinea grass and fertilized with 0, 70, 140 and 210 kg ha$^{-1}$ of N.

| N rate (kg ha$^{-1}$) | Species | | | Average |
|------|------|------|------|------|
| | *U. ruziziensis* | *U. brizantha* | *P. maximum* | |
| ------------------------------------------- | | Kg ha$^{-1}$ | -------------------------------------------------- | |
| 0 | 26 | 29 | 37 | 30 d |
| 70 | 54 | 61 | 77 | 64 c |
| 140 | 100 | 106 | 103 | 103 b |
| 210 | 150 | 153 | 173 | 159 a |
| Average | 83 B | 87 B | 98 A | 89 |

585 Average followed by different letter, lowercase in the column and uppercase in the row, differ
586 at the 5% probability level (Tukey, $p<0.05$).

587

588

589 **Table 3.** Partial nitrogen balance in cropping systems where maize fertilized with 0, 70, 140
590 and 210 kg ha$^{-1}$ of N was cropped after ruzigrass, palisade grass and Guinea grass cultivated
591 for 11 months.

| N rate | Species | | | Average |
|--------|---------|---------|---------|---------|
| | *U. ruziziensis* | *U. brizantha* | *P. maximum* | |
| ---------------------------------------------- Kg ha$^{-1}$ ---------------------------------------------------- | | | | |
| 0 | -84 | -86 | -94 | -88 d |
| 70 | -42 | -49 | -65 | -52 c |
| 140 | -18 | -24 | -20 | -21 b |
| 210 | 2 | -1 | -21 | -7 a |
| Average | -36 A | -40 A | -50 B | -42 |

592 Different letters, lowercase in columns and uppercase in rows, show significant differences
593 (Tukey test, *p*<0.05).
594